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GLUCOSE METABOLISM OF FED, STARVED
AND TOXAEMIC PREGNANT SHEEP

A thesis submitted in fulfilment of
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by

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Glucose Metabolism of Fed, Starved and Toxaemic Pregnant Sheep.

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Glucose metabolism was studied in fed, starved and toxaemic ewes in the last month of gestation to ascertain causes of hypoglycaemia, and to determine whether the glucose metabolism of sheep susceptible to ovine pregnancy toxaemia (OPT) differs from those not susceptible.

Glucose kinetics were measured with a single injection of [U-¹⁴C], [6-³H]glucose. Subsequently glucose tolerance tests were performed using 0.4 g glucose/kg liveweight.

Ewes were starved for 10 days to induce OPT, and blood samples were taken from one jugular vein and analysed for packed cell volume, glucose, ketones, free fatty acids, urea, creatinine, inorganic phosphate, total CO₂, total plasma protein and albumin. Glucose turnover was measured by a primed continuous infusion of [U-¹⁴C], [6-³H] glucose when the ewes became recumbent with OPT (and these were classified as susceptible, S) otherwise at the end of 10 days (and these were classified as non-susceptible, NS). The ewe was slaughtered at the end of the infusion. The caudate lobe of the maternal liver was removed, and perfused with collagenase to prepare hepatocytes for measuring the gluconeogenic potential of the liver.

In another experiment, the effect of live fetuses on maternal glucose metabolism was assessed. Nooses were surgically implanted around the umbilical cords of fetuses in twin-pregnant ewes in the last month of gestation. Ten days after surgery, the ewes were starved for four days. Glucose turnover was measured by continuous infusion of [U-¹⁴C], [6-³H] glucose and then the fetuses were killed by tightening the nooses. Blood samples were taken.

for 24 hours, to monitor glucose and acetoacetate concentrations. The ewe was then given a second infusion of tracer glucose, and subsequently killed for hepatocyte studies.

A 5-compartment model was proposed for glucose kinetics of fed, twin-pregnant ewes. Data of S and NS ewes were compared by fitting to the model separately, using the SAAM program.

Ewes susceptible to OPT had significantly higher insulin resistance ($2043 \mu\text{U/ml. min}$) than NS ewes ($1261 \mu\text{U/ml. min}$). This criterion could be useful in screening sheep for susceptibility before inducing the disease, and also provides evidence that OPT may be related to a poor ability to maintain glucose homeostasis.

During starvation plasma glucose decreased, while ketones and free fatty acids increased. The final concentrations were related to the state of the lambs rather than OPT symptoms. Indications of renal failure (raised serum creatinine, urea and inorganic phosphate, and lowered total CO_2) were observed in both S and NS ewes. On post-mortem analysis, liver glycogen of S ewes was 10% (2 mg/g) the level of NS ewes (18 mg/g).

The gluconeogenic potential of hepatocytes from S ewes, with the substrates propionate, lactate, alanine, glutamine and glycerol, was significantly lower than that of NS ewes and the rates were not stimulated by glucagon. It is hypothesized that the development of OPT may be related to a lesion in glucose production.

Ewes with dead lambs were often hyperglycaemic (plasma glucose greater than 80 mg/dl), had significantly higher glucose turnover rates (6.90 g/kg/day) than those with live lambs (2.67 g/kg/day), and had significantly higher gluconeogenic potentials, measured on hepatocytes. This suggested that the maternal glucose production was restricted by the presence of live fetuses.

Killing the foetuses in utero raised plasma glucose (from 27 to 99 mg/dl), increased glucose turnover (from 2.39 to 4.82 g/kg/day) and significantly increased hepatocyte glucose production. It is hypothesized that the hypoglycaemia of starved pregnant ewes is due to inhibition of hepatic glucose production by some foeto-placental factor.

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CHAPTER 1

LITERATURE REVIEW

1. INTRODUCTION

Ovine pregnancy toxaemia (OPT) is mainly a disease of multi-foetate ewes in the latter stages of gestation, and it is characterized by hyperketonaemia, hypoglycaemia and often on post-mortem, a fatty liver. Factors contributing to the disease are undernutrition and stress.

In New Zealand pregnancy toxaemia may account for 10% of ewe deaths (Davis, 1974), which can amount to 135,000 per year in the national flock of 45 million breeding ewes. The economic loss is substantial (in excess of \$2 million/year) and could rise as sheep are selected for increased fecundity and a higher proportion are multi-foetate.

Although the disease has been studied for a long period of time - since it was first documented by Seaman in 1855 - its pathogenesis is still only partially understood, and consequently therapeutic measures usually produce few recoveries.

The literature on OPT and carbohydrate metabolism in sheep is reviewed prior to describing the experiments undertaken, and for the purposes of this text, 'starved' refers to starved non-pregnant sheep, 'ketotic' refers to starved pregnant sheep not showing OPT signs, 'OPT sheep' refers to those showing clinical symptoms while the term 'toxaemic' is reserved for ewes showing terminal signs of the disease.

2. PREGNANCY TOXAEMIA

2.1 DEVELOPMENT OF THE DISEASE:

Prior to 1940 many factors, such as infection, mineral deficiency, brain or kidney damage and high protein intake were suggested as causes of OPT (Groenewald et al., 1941b). While these theories lacked experimental support, others based on number of foetuses, lack of exercise, overfatness, choline and vitamin deficiency have not been well researched. The effect of pregnancy, level of nutrition and stress on the development of the disease have been studied and are considered to be predisposing factors.

2.1.1 PREGNANCY

OPT has been induced in non-pregnant ewes by suddenly decreasing the ration of obese sheep (Clark, 1943) and by severely underfeeding for 24-48 days (Groenewald et al., 1941a). This led to the suggestion that pregnancy was not a necessary factor for the development of toxaemia but just hastened its onset. However the latter observations were made on sheep after prolonged semi-starvation, and so may not have represented the condition which arises during pregnancy. Furthermore, Fraser et al. (1939) failed to induce the condition in non-pregnant ewes, and it is now generally agreed that pregnancy is a necessary condition for the development of OPT. Abortion alleviates the condition (Hunt, 1976) and this implicates the foetus, and hence pregnancy, in the pathogenesis.

2.1.2 LEVEL OF NUTRITION:

While sheep maintained on a high level of nutrition, or changed from a low to a high level, do not develop OPT (Gill and Thomson,

1954; Fraser et al., 1939), controversy exists over the effects of a continuous low level of nutrition, since some have found that it induced the disease (Thomson and Thomson, 1949) while others have not (Wallace, 1948; Coop, 1950).

Reducing the food intake towards the end of pregnancy increased the incidence in all cases (Blaxter, 1957; Gill and Thomson, 1954).

2.1.3 STRESS:

Stress in the form of sudden climatic or environmental change can precipitate the disease. Since stress influences adrenal activity (Reid, 1962; Reid and Mills, 1962), OPT may be associated with the adrenal response to stress, and several theories are discussed in Section 2.4.

2.2 BEHAVIOURAL AND CLINICAL SYMPTOMS:

The clinical symptoms observed by McClymont and Setchell (1955a, 1956b) followed a sequence, and were initially mild and intermittent but became more severe and progressed eventually to coma and death.

(i) Depression of Consciousness:

Initially the affected sheep became slow in movement and lagged behind the flock when moved. They were easily approached and often stood in unusual positions such as in water troughs, or with their head propped against a fence or in a corner.

(ii) Depression and Loss of Eye Preservation and Auditory Reflexes:

The affected sheep then became less conscious of their environment, and walked into obstacles. Ear and eye reflexes were lost, but hypersensitivity occasionally occurred for short periods.

(iii) Myoclonus:

Spasmodic twitching of periorbital and aural muscles occurred, and occasionally those of the trunk and limbs. This produced fits which lasted several minutes. Champing and chewing movements were also observed.

(iv) Postural and Locomotory Disturbances:

Clonic contractions led to staggering movements, and the sheep were seen walking in circles, and on recovery were found sitting or lying in unnatural positions.

(v) Sternal or Lateral Recumbency:

Initially the affected sheep became recumbent sternally, but rose if prompted. This condition generally progressed to lateral recumbency, as the sheep became comatose.

(vi) Excretory and Secretory Changes:

Scouring often occurred in the latter stages of the disease, and excessive nasal secretion of mucous was obvious.

(vii) Death:

Death sometimes occurred within a few hours of lateral recumbency, or was delayed for some days after the sheep had reached this condition.

Appetite was generally lost early in the development of the disease (Van Rensburg, 1931).

Reid (1968) differentiated three syndromes based on prior nutrition, clinical signs, prognosis and metabolite changes. The first syndrome was seen in prolonged and severely undernourished ewes, such as those maintained experimentally on a constant ration.

It occurred when feed was the main limiting factor. Acidosis and renal failure were evident from the early stages of the disease, and were accompanied by marked hypoglycaemia and ketosis. Coma and death occurred relatively quickly.

The second syndrome was less severe and was observed when underfeeding was accompanied by a stress, such as disease or bad weather. It also occurred when previously undernourished ewes were fasted and was considered to be the most common form seen in the field. This syndrome occurred therefore, after prolonged undernutrition.

The third syndrome was observed when well-nourished ewes were suddenly underfed. It occurred when ewes were experimentally fasted, and in the field when ewes were mustered, yarded or trucked. The symptoms were generally mild, and according to Reid (1968) these cases responded to glycerol therapy. This syndrome may include the condition seen in fat ewes in the midst of apparent plenty, and termed 'idiopathic OPT' (Pugh, 1954). It was considered to be the result of appetites of fat ewes being more easily depressed in stressful situations. This makes them more susceptible to OPT (see Reid, 1968).

Experimentally starving ewes in the latter stages of gestation produced symptoms similar to those seen in the field (Parry, 1950). This induced condition was considered by Reid (1968) to be the same as OPT observed in syndrome 3; however Thomson (1956) observed that in induced cases ACTH produced a clinical recovery and glycerol caused a severe hyperglycaemia, while these effects were not observed in field cases.

2.3 METABOLITE AND PATHOLOGICAL CHANGES:

2.3.1 METABOLITE CHANGES IN THE BLOOD:

Pregnancy toxæmia is characterized by hypoglycaemia, hyperketonaemia and increased plasma concentrations of free fatty acids. The packed cell volume may also rise (Kronfeld, 1972). However these changes were similar in both ketotic and OPT sheep (O'Hara et al., 1975). These features of the disease were no different in degree to changes which occurred on starvation, and furthermore they were reversible on refeeding in sheep not showing signs of OPT.

Early studies compared the metabolite levels in sheep with OPT, with those found in normal sheep. No differences were observed in the serum minerals Ca^{++} , Mg^{++} , Na^+ or K^+ (McClymont and Setchell, 1955a), Cl^- (Ferris et al., 1969), phosphorus (see Groenewald et al., 1941b) or cholesterol (Roderick et al., 1933). In the study of Ferris et al. (1969) the plasma protein, fibrinogen, the tricarboxylic acid cycle intermediate, pyruvate, and the acid-base status were not altered by the disease. This study differs from others in that they found no change in packed cell volumes but did observe some proteinuria.

These kinds of observations have limited value in distinguishing OPT, as the severity of the changes could be due to differences in prior nutrition and in the number of foetuses carried (Reid, 1968).

To overcome these problems and to allow for a better definition of the disease, some workers (O'Hara et al., 1975) adopted a metabolic profile to monitor the development of the disease. The sheep were fed the same rations and were differentiated according to number of foetuses and metabolite levels, when monitored throughout a starvation period. This allowed a comparison to be made of ketotic and

OPT sheep, and the noting of changes which occurred with the development of toxaemia.

The metabolite changes noted were: decreases in blood glucose and increases in blood ketones and packed cell volume. No changes were noted in Na^+ , but there were decreases in K^+ and Ca^{++} . The variable concentrations of plasma protein and albumin were little different in either ketotic or OPT sheep (O'Hara et al., 1975).

Some OPT sheep did develop toxaemia and showed marked changes in their plasma composition. When renal failure occurred, urea, creatinine and phosphate concentrations increased, and acidosis was marked by decreased HCO_3^- levels. Often a terminal hyperglycaemia developed, which may have been a consequence of renal failure and/or foetal death (O'Hara et al., 1975).

One obvious feature of the disease was that despite the controlled conditions, there was considerable variation, between sheep, in the changes of particular metabolites (those of glucose, ketones, and free fatty acids) during starvation. This suggests that there may be differences in the ability of sheep to adapt to starvation.

Relationship of biochemical changes to clinical symptoms:

OPT is characterized by hypoglycaemia and hyperketonaemia, but the association between these biochemical changes and other clinical symptoms is not well defined. No relationship has been found between the levels of glucose or ketones, and the appearance of neurological symptoms (Parry, 1950; Gill and Thomson, 1954; Reid, 1960b; Ferris et al., 1969). The normal range of plasma glucose in fed ewes is 45-60 mg/dl, and Reid (1960b) recorded plasma glucose concentrations in ketotic ewes of 12-31 mg/dl, in ewes with OPT induced by starvation, 10-56 mg/dl, and in field cases of OPT

7-72 mg/dl. Ketones (mg acetone/dl) ranged from 8-70 mg/dl in the ketotic ewes, 9-95 mg/dl in induced OPT ewes and 8-86 mg/dl in field cases. While the results may have been influenced by the degree of undernutrition and the number of foetuses carried (Reid, 1960b; 1968), there was no apparent diagnostic glucose or ketone level.

The appearance of symptoms is generally preceded by a period of hypoglycaemia and hyperketonaemia, and there is a relationship between the depth of hypoglycaemia and their development (McClymont and Setchell, 1955a; 1956a). The plasma glucose concentration of ketotic ewes, 20.1 ± 1.1 mg/dl, and OPT ewes prior to the development of neurological symptoms, 15.6 ± 1.0 mg/dl were significantly different (McClymont and Setchell, 1956a). Thus hypoglycaemia was considered to have a role in the induction of the syndrome, but was not alone responsible for the cerebral depression, as hyperglycaemia sometimes developed after the appearance of symptoms with no clinical improvement (Reid, 1960b). This implied that irreversible brain damage had occurred (McClymont and Setchell, 1955b).

A relationship has also been established between the degree of the preceding hyperketonaemia and the severity of the symptoms (Reid, 1960b).

In summary, a period of hypoglycaemia precedes the development of OPT symptoms, and glucose shortage is thought to be one of the primary metabolic lesions, but at the time neurological symptoms appear there is no diagnostic glucose or ketone level.

2.3.2 METABOLITE CHANGES IN THE LIVER:

Glycogen levels of affected ewes were very low (Snook, 1939; Underwood et al., 1943) and while, on a fresh weight basis, the level of glycogen in fed pregnant ewes was 3.8%, in ewes with OPT it was 0.3% (Roderick et al., 1933).

On post-mortem, livers from ewes with OPT are characteristically fatty in appearance, and on the basis of dry weight, crude fat was 60% in OPT sheep and 7% in fed pregnant sheep (Roderick et al., 1933). However levels were also elevated in ketotic ewes, and no difference was detected between ketotic and OPT sheep (Snook, 1939). Thus a fatty liver is not a characteristic unique to OPT.

Dye-excretion tests were impaired in OPT indicating that liver function or hepatic blood flow was decreased (Roderick et al., 1933). However data which compare liver function of ketotic and OPT sheep have not been obtained.

2.3.3 CHANGES IN KIDNEY FUNCTION:

Renal impairment has been observed early in the disease (McCausland et al., 1974; Wolff, 1977) and it increased in severity as the disease developed (Parry and Taylor, 1956). Renal blood flow and glomerular filtration rate were both reduced in OPT sheep when compared to fed sheep (Kaufman and Bergman, 1978) but these workers did not obtain data from toxæmic sheep (Wolff, pers. comm.). Urea and creatinine clearances were reduced in sheep with OPT, compared to fed sheep (Parry and Taylor, 1956) and these changes also indicate renal impairment.

Generally no change has been recorded in the blood pressure of sheep with OPT (Parry and Taylor, 1956), and so in contrast to the human condition, hypertension and oedema would not seem to be features of ovine pregnancy toxæmia.

2.3.4 PATHOLOGICAL CHANGES:

The main pathological change was generally a fatty, friable liver (Groenewald et al., 1941a). The kidneys were pale (McCausland et al., 1974) and while glomerular changes have been noted (Ferris et al., 1969) these may have been due to an unrelated kidney disease, as normally no lesions or ultrastructural changes were obvious (McCausland et al., 1974).

The adrenals were often enlarged (Groenewald et al., 1941a; Reid, 1960c), but not always (Ferris et al., 1969). No lesions have been detected in the brain (McCausland et al., 1974), heart or lungs (Groenewald et al., 1941a).

2.4 ENDOCRINE CHANGES:

2.4.1 CORTISOL:

Cortisol secretion from the adrenal cortex is stimulated by ACTH from the pituitary, in response to stresses such as starvation, hypoglycaemia, emotional disturbances and wet and cold atmosphere (Reid, 1962; Reid and Mills, 1962). Metabolically, cortisol inhibits glucose utilization (Bassett et al., 1966), and stimulates gluconeogenesis (see Reid, 1968).

The plasma cortisol levels were generally increased in field cases of OPT (1-10 $\mu\text{g}/\text{dl}$) compared to ketotic ewes (less than 3.5 $\mu\text{g}/\text{dl}$), and as the adrenals were hypertrophic (6.7 g) compared to normal ewes (3.8 g), this suggested that the secretion rates were increased (Reid, 1960c). On the basis of these and earlier observations, Reid and Hogan (1959) and Reid (1960c) compared OPT to a hyperadrenal condition. They considered that the stress of prolonged undernutrition, hypoglycaemia and adverse environment caused

excess cortisol release. The cortisol inhibited glucose utilization and stimulated glucose production. However the foetus took up the glucose produced. This sustained the hypoglycaemia, and together with the inhibited glucose utilization led to cerebral depression, and toxæmia. They called this condition of field cases, corticosteroid diabetes. This theory was not supported by Saba and Cunningham (1971), who, in a study of field cases of OPT, found that cortisol levels were elevated, but only to the same degree as in sheep suffering from hypocalcaemia, and they concluded that high concentrations of plasma cortisol do not necessarily indicate that hyperadrenal activity is the cause of the disease.

In contrast to the field cases, Saba et al. (1966) observed that cortisol levels were not always elevated in induced cases, and that the administration of ACTH to stressed, fasted ewes actually prevented the condition. To explain these observations, Reid (1968) proposed a theory of adrenal cortical insufficiency, which postulates that in the subacute form of the disease (i.e. syndrome 3, where the disease developed after the ewes were fasted and stressed), the hypoglycaemia, and OPT develop due to insufficient response by the adrenal cortex. Direct evidence to support this theory has not been obtained.

2.4.2 INSULIN:

Plasma insulin-like activity (ILA) is determined by a bioassay technique which measures the effect of insulin on glucose uptake by rat diaphragm or adipose tissue (Cunningham, 1962a). This method measures the net difference between factors which increase, and antagonize, glucose uptake and the results are generally higher than the more specific radioimmunoassay method (Dash and Lindsay, 1967).

ILA levels fell when pregnant ewes were fasted, from about 300 to 50 μ U/ml (Saba et al., 1966). However, while Leng (1965) recorded high ILA levels in some ewes with OPT (up to 1851 μ U/ml), Cunningham (1962b) found that generally the levels were reduced. No data are available for insulin determined by radioimmunoassay, during OPT.

Ewes with OPT seemed to be resistant to insulin, as insulin infusions did not increase the hypoglycaemia (Reid, 1960d).

2.4.3 GLUCAGON:

Glucagon is an important hormone in maintaining glucose homeostasis by stimulating gluconeogenesis and glycogenolysis (Brockman and Bergman, 1975a). However no data exist for OPT sheep on the levels of glucagon, or the insulin/glucagon ratio, which is an indicator of the relative actions of the hormones (Unger, 1971).

2.4.4 OTHER HORMONES:

The level of growth hormone, which increases during starvation (Bassett and Madill, 1974) or thyroxine, which regulates metabolic rate, has not been reported for either ketotic or OPT sheep.

Adrenaline and noradrenaline are released in response to stress, and both are increased in the plasma (Thompson et al., 1978), and urine (Webster et al., 1969) following cold stress. Adrenaline release, measured indirectly, is increased in sheep during insulin-induced hypoglycaemia (Setchell and Waites, 1962), although Saba et al. (1966), using a non-specific method found no increase in plasma catecholamines during the stress of fasting and trucking.

In conclusion, few hormones have been monitored in OPT, and of those that have, comparisons are lacking between ketotic, OPT and toxaemic sheep.

2.5 ENZYMOLOGY OF GLUCONEOGENESIS IN RELATION TO OPT:

The four enzymes which are considered to limit the rate of gluconeogenesis are glucose-6-phosphatase (EC 3.1.3.9), fructose-1,6-biphosphatase (EC 3.1.3.11), pyruvate carboxylase (EC 6.4.1.1) and P-enolpyruvate carboxykinase (EC 4.1.1.32) (Bergman, 1973). The activities of these enzymes are altered by diet, fasting, pregnancy, and renal failure and the changes are probably hormonally mediated. Glucose-6-phosphatase, fructose-1,6-biphosphatase and pyruvate carboxylase all increase during starvation, but the activity of P-enolpyruvate carboxykinase was not increased in either starvation or ketosis (Filsell et al., 1969). The levels of these four enzymes have not been compared in ketotic and OPT sheep.

The endogenous respiration, and oxidation of volatile fatty acids by liver slices was not changed in ketotic sheep (Smith et al., 1961). However liver mitochondria from OPT sheep were unable to oxidize octanoate or palmitate (Gallagher, 1959).

The levels of nicotinamide coenzymes in the liver have been compared in fed, ketotic and field cases of OPT (Kronfeld and Raggi, 1966). NAD and NADH₂ are involved in fatty acid oxidation and were reduced in both ketotic and OPT sheep. The differences in oxidation of long chain fatty acids by ketotic and OPT sheep observed by Gallagher (1959) could not therefore be explained by a reduction of this coenzyme.

The coenzymes, NADP and NADPH₂, are involved in fatty acid synthesis and were reduced only in sheep with OPT (Kronfeld and Raggi, 1966). They suggested that a nicotinamide deficiency could account for the lack of adenine nucleotide coenzymes in ketotic

ruminants, even though the vitamin (now called niacin) is synthesized by rumen microbes from tryptophan.

2.6 THERAPY:

Ewes with OPT if left untreated have recoveries of 20 % (Thomson, 1956), and consequently many therapies have been tried in an attempt to improve this figure.

Natural abortion aids recovery (Gill and Thomson, 1954) and inducing parturition by dexamethasone raised survival to 95 % (Hunt, 1976). Although it cannot be considered to be an ideal treatment, due to the subsequent lamb losses, it may be expedient for saving the ewe, for if the lamb dies in utero and is not aborted the ewe generally becomes hyperglycaemic and dies of toxæmia (Reid, 1960d; Clark et al., 1943).

Glucose administered orally, subcutaneously or intravenously, is generally ineffective (Hopkirk, 1934; Cameron and Goss, 1940; Thomson, 1956). However twice daily injections of 2.5 ml of 60 % glucose solution enabled 75 % of treated ewes to recover (McClymont and Setchell, 1955b).

Several glucose precursors have also been tested, and propylene glycol (under the tradename 'Ketol') is used commercially. Glycerol, given orally, while alleviating biochemical symptoms of OPT, produced no clinical improvement (Reid, 1960a). However daily dosing of 3 ml/kg did raise recoveries from 17 % to 78 % (McClymont and Setchell, 1955b). Glycerol was mildly effective alone but increased survival of treated ewes to 50 % when supplemented with insulin or ACTH (Thomson, 1956). The effectiveness of glucose and its precursors seemed to depend on the stage of the disease at which they were given, and early treatment with frequent dosing was often successful.

Corticosteroids and ACTH have been frequently recommended as treatments but have had little success. Cortisone and cortisol did not improve recoveries, and ACTH, although it tended to cause abortions, had no beneficial effect (Holm, 1958; Thomson, 1956). A 61% recovery was achieved though when field cases were injected with a mixture of the corticosteroids prednisolone and dexamethasone TMA (Hazzard and Russell, 1968). Additional supportive therapy such as oral glycol, glucose, dextrose and injections of Ca/P/Mg lifted the recovery to 85%, but again these results also included recoveries due to abortions (Hazzard and Russell, 1968).

Results with another adrenal hormone, adrenaline, have been conflicting. Thomson and Thomson (1949) reported recovery of early cases while Hopkirk (1934) claimed it was ineffective as a treatment.

Of the pancreatic hormones, intravenous injection of insulin reduced the plasma levels of ketones and glucose (Reid, 1960d) and was ineffective as a treatment (Hopkirk, 1934). Glucagon raised glucose and reduced ketone levels of ketotic ewes (Ho and Reber, 1957) but the results were transitory and it has not been assessed in sheep with OPT.

The anabolic steroid trenbolone acetate gave a good recovery of 77% (Heitzman et al., 1977) and had the advantage of stimulating appetite.

A therapy claimed to give good results was oral dosing with bonemeal (Van Rensburg, 1931). However the salts calcium chloride and sodium bicarbonate were ineffective (Hopkirk, 1934), and these observations were consistent with the constant levels of serum minerals in OPT (McClymont and Setchell, 1955a). The disease diagnosed by Van Rensburg (1931) may therefore have been hypocalcaemia.

A common defect in therapeutic trials has been the lack of objective criteria for assessing the degree of toxaemia before therapy is begun. In the early stages of OPT symptoms, treatment of ketosis and hypoglycaemia may be sufficient; however when the toxaemic symptoms of anorexia, renal failure, acid-base imbalance and hyperglycaemia occur, different strategies are required to correct these conditions.

3. CARBOHYDRATE METABOLISM OF SHEEP

3.1 INTRODUCTION

Glucose is derived from three sources in the ruminant - absorption from the gut, synthesis by the liver and synthesis by the kidney.

While little or no glucose is absorbed from the digestive tract on roughage diets, small amounts may be absorbed on grain diets (Sutton and Nicholson, 1968) but this has not always been observed (Bergman et al., 1970). It is generally agreed that in most fed situations and certainly in starvation the animal must rely entirely on gluconeogenesis for its glucose needs (Bergman, 1973).

Using arterial-venous difference techniques it has been established that the liver produces 85% of the glucose used by the sheep and the kidney produces 10%. The ratio did not change in pregnancy, but the renal contribution increased slightly in starvation (Bergman, 1973). Thus the sheep is heavily dependent on hepatic gluconeogenesis for its glucose.

Glucose metabolism in the ruminant has been the subject of several reviews. Those of Reid (1968), Leng (1970) and Bergman (1973) refer primarily to sheep while that of Young (1977) refers to cattle. Ballard et al. (1969) reviewed and contrasted fat and glucose metabolism of ruminants and non-ruminants.

3.2 HEPATIC GLUCOSE SYNTHESIS

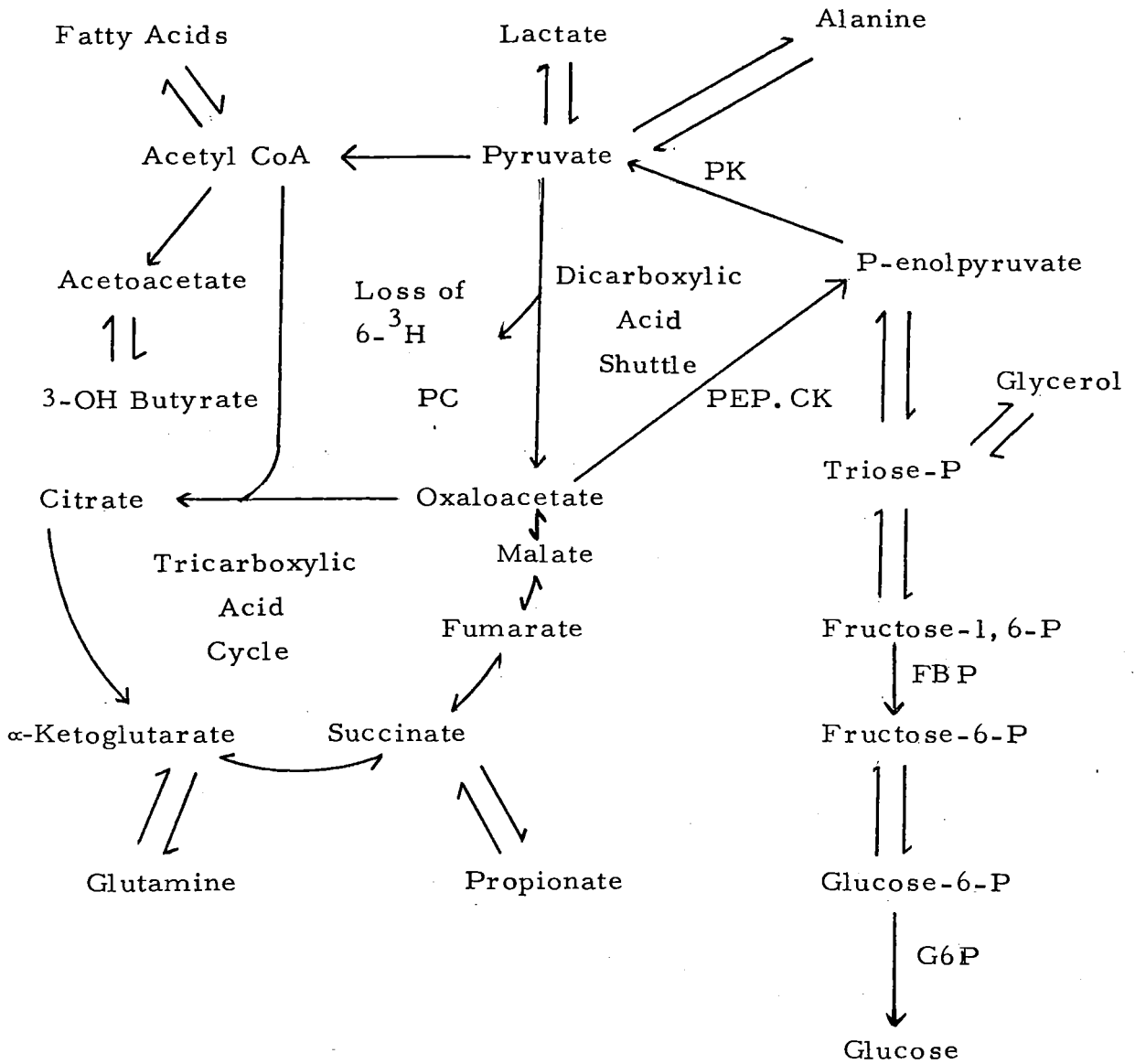
3.2.1 PATHWAY AND ENZYMES:

With the exception of four enzymes, glucose synthesis is principally by a reversal of the glycolytic pathway (Fig. 1), as the same enzymes involved in glycolysis catalyse the reversible reactions. The four irreversible steps involve enzymes specific for gluconeogenesis. The activities of these enzymes in sheep (as the rate CO_2 is fixed, or phosphate released per g liver wet weight) are double those of the rat (Wagle and Nelson, 1966) and this is considered to reflect the relative importance of gluconeogenesis in these species. However, in sheep the activities are still lower than other enzymes in the pathway and they are thus considered to be potentially rate limiting.

These four potentially limiting enzymes are glucose-6-phosphatase (EC 3.1.3.9), fructose-1,6-biphosphatase (EC 3.1.3.11), pyruvate carboxylase (EC 6.4.1.1) and P-enolpyruvate carboxykinase (EC 4.1.1.32) (Bergman, 1973). A fifth enzyme, pyruvate kinase (EC 2.7.1.40) decreases gluconeogenesis by causing a futile cycle between phosphoenolpyruvate and pyruvate (see Fig. 1).

The activities of these enzymes can be altered by the type of diet, starvation, physiological condition of the animal, such as pregnancy, lactation or exercise, and by disease such as renal failure. The changes may be hormonally mediated but in contrast to non-ruminants enzyme regulation has not been well researched in the ruminant.

Glucose-6-phosphatase releases free glucose into the blood and this enzyme is found only in the liver and kidney. It may be



Enzymes:

- PC = pyruvate carboxylase
 PEP.CK = P-enolpyruvate carboxykinase
 PK = pyruvate kinase
 FBP = fructose-1,6-biphosphatase
 G6P = glucose-6-phosphatase

Fig. 1: Pathway of gluconeogenesis and glycolysis

under strong hormonal regulation as insulin inhibits glucose release, and glucagon and adrenaline stimulate it (Bassett, 1971; West and Passey, 1967). The reverse process of glucose uptake is performed by hexokinase and a specific enzyme, glucokinase; however the level of these enzymes was low or absent in the ruminant liver (Ballard et al., 1969) so glucose is mainly taken up by extrahepatic tissues.

Pyruvate carboxylase regulates the formation of oxaloacetate from pyruvate and so controls gluconeogenesis from pyruvate, lactate and alanine. As its activity is increased by starvation and glucagon, and decreased by cortisone and synthetic glucocorticoids (Filsell et al., 1969; Brockman and Manns, 1974) this enzyme responds to dietary and hormonal control.

P-enolpyruvate carboxykinase converts oxaloacetate to phosphoenolpyruvate and so it controls gluconeogenesis from all precursors except glycerol.

Pyruvate kinase is involved in glycolysis and its activity is higher in animals on concentrate diets (Pearce and Unsworth, 1976). Alternatively it may be inhibited on roughage diets to maximize glucose production. The ratio of P-enolpyruvate carboxykinase to pyruvate kinase has been considered to represent the liver's potential for gluconeogenesis (Edwards et al., 1975).

3.2.2 PRECURSORS:

The main glucose precursors in ruminants are propionate, lactate and pyruvate, glycerol and amino acids (Bergman, 1973).

Propionate is the only volatile fatty acid produced in the rumen capable of increasing the net output of glucose. Acetate and

butyrate are metabolized via the tricarboxylic acid cycle and so their carbon contribution is offset by the loss of two CO_2 molecules.

The rate of gluconeogenesis (irreversible loss) is proportional to the rate of propionate production in the rumen, and this is related to the diet (Steel and Leng, 1973b). The rate of propionate production also affects the percentage of glucose derived from propionate (Steel and Leng, 1973b).

Based on hepatic uptake studies, propionate could maximally contribute 40% of the glucose synthesized by the liver (Bergman, 1973). Estimates based on conversion rates of [^{14}C] propionate to glucose underestimate the contribution as they do not account for crossing over of label in the tricarboxylic acid cycle (Krebs *et al.*, 1966).

Normally little L+ lactate is produced in the rumen. Most arises from anaerobic metabolism of glucose, especially in the muscle after exercise. When this is resynthesized it makes no net contribution to the glucose pool. This cycle through the lactate pool is called the Cori Cycle.

In fed sheep it has been estimated by different techniques, to contribute 4-6% of glucose turnover (Annison *et al.*, 1963; Brockman *et al.*, 1975b). The amount recycled through lactate was not altered by starvation, glucagon or dexamethasone treatment (Brockman *et al.*, 1975b). An interrelationship has been shown in sheep whereby the rate of conversion of glucose to lactate depends on the glucose concentration, and the rate of lactate conversion to glucose depends on the lactate concentration (Reilly and Chandrasena, 1978). These results suggest that recycling may be increased in hyperglycaemia. Normally about 15% of the glucose pool is derived from lactate (Annison *et al.*, 1963).

Glycerol is bound to fatty acids as triglycerides and only a small amount exists as free glycerol in the plasma. In the fed, non-pregnant ewe it contributes 5% of the glucose turnover, but its importance as a precursor increases during starvation and hypoglycaemia, when mobilization of fat releases free glycerol (Bergman et al., 1968).

Amino acids may originate from protein in the muscle, or from dietary proteins and are catabolized by the liver and kidney. Just as lactate shuttles energy between the liver and tissues, amino acids are considered to have a role in transporting carbon and nitrogen. An alanine cycle has been proposed in the human (Felig et al., 1970) to link amino acids with gluconeogenesis, and in sheep glutamine seems important in transporting carbon and nitrogen between organs (Heitmann and Bergman, 1978). Using arterio-venous difference techniques to give the maximum amount of glucose synthesized from amino acid uptake, and labelled amino acid conversion to glucose to give the minimum, Wolff and Bergman (1972) calculated that amino acids contributed between 11-30% of glucose turnover. Alanine and glutamate were the main gluconeogenic amino acids.

The contribution of amino acids to glucose is expected to increase during starvation and this has been demonstrated after three days starvation (Heitmann and Bergman, 1978) for glutamine.

3.3 REGULATION OF GLUCOSE SYNTHESIS

This section reviews factors which regulate glucose synthesis in the normal fed animal. (Changes that occur in pregnancy and starvation are reviewed in subsequent sections.)

Glucose synthesis is influenced by factors outside the liver, which control the supply of precursors, and in the liver by the rate-limiting steps in the synthetic pathway. Hormones affect gluconeogenesis through precursor availability, and enzyme activity.

3.3.1 PRECURSOR SUPPLY:

Precursors are derived exogenously from the rumen, and endogenously by tissue mobilization and synthesis from other compounds.

The supply of exogenous precursors is largely controlled by the diet. The rate of gluconeogenesis is related to concentration, and rate of production, of propionate in the rumen (Steel and Leng, 1973b) and is also influenced by dietary protein intake, and the rate of amino acid entry into plasma (Reilly and Ford, 1971). Infusing protein into the abomasum stimulated gluconeogenesis (Judson and Leng, 1973b).

As digestible organic matter directly affects the availability of ruminal propionate and amino acids, glucose synthesis depends on the quantity and quality of diet in the non-pregnant sheep (Steel and Leng, 1973a).

The incorporation of glycerol and lactate into glucose increases with their entry rates (Bergman et al., 1968; Reilly and Chandrasena, 1978) but this does not mean they stimulate gluconeogenesis per se.

Additional evidence for precursors influencing gluconeogenesis is that glucose production increases threefold in lactation and food intake increases threefold (Bergman and Hogue, 1967).

Conversely glucose entry rate decreases on starvation, when exogenous precursors are reduced (Annison and White, 1961; Bergman, 1963).

Appetite has been suggested to affect glucose synthesis, probably through precursor supply (Leng, 1970). The area of the hypothalamus which regulates appetite may be affected by changing metabolite levels, possibly acetate, in the rumen (Baile, 1968).

3.3.2 HORMONAL REGULATION OF PRECURSOR SUPPLY:

The effect of diet on hormones regulating carbohydrate metabolism has been reviewed by Bassett (1975). The pancreatic hormones insulin and glucagon are considered to be the main regulators. Those of the adrenal cortex (cortisol and cortisone), the intestine (secretin and pancreozymin), the pituitary (growth hormone and adrenocorticotrophic hormone), the adrenal medulla (adrenaline and noradrenaline) and thyroxine are all postulated to play a role in glucose metabolism but their effects may be mediated by insulin and glucagon.

3.3.2.1 Insulin:

The action of insulin in ruminants has been reviewed by Trenkle (1972) and the actions of insulin and glucagon by Brockman (1978).

Insulin stimulates glucose uptake and utilization by peripheral tissues (West and Passey, 1967) and also inhibits glucose release from the liver (West and Passey, 1967; Brockman et al., 1975b). These actions, combined, produce hypoglycaemia. As glucose production was only slightly inhibited (West and Passey, 1967) and gluconeogenesis from alanine inhibited 5%, it is considered that

insulin has a greater effect on glucose use by peripheral tissues than on hepatic glucose synthesis (Brockman, 1978).

Insulin alters protein metabolism by stimulating amino acid uptake at extrahepatic sites, and this results in lower plasma amino acid concentrations (Brockman et al., 1975a). It does not affect hepatic uptake of amino acids (Brockman et al., 1975a) or incorporation of alanine into glucose (Brockman, 1978).

In vitro studies with isolated fat cells have shown that insulin stimulates lipogenesis and inhibits lipolysis (Yang and Baldwin, 1973a, b). These observations are supported in vivo in that insulin infusions decrease plasma glycerol (Bergman, 1968) and glycerol release (Brockman et al., 1975a), and also decrease plasma free fatty acids and their release (West and Passey, 1967).

While in non-ruminants the products of digestion (especially glucose) have a marked effect on insulin release, in ruminants the mode of digestion makes such relationships less obvious.

Although intravenous infusion of glucose causes an increase in plasma insulin (Brockman, 1977) the correlation between plasma glucose levels and insulin concentration is low (Bassett et al., 1971). This suggests that plasma glucose concentration may not be the most important regulator of insulin concentration.

The rise in insulin levels after feeding (Ross and Kitts, 1973) together with the stimulation of insulin levels after infusion of volatile fatty acids (Horino et al., 1968) led to the hypothesis that volatile fatty acids are important regulators of insulin secretion in ruminants. This was supported by Brockman (1978) but the physiological importance has been questioned (Stern et al., 1970; Trenkle, 1972; Bassett, 1975) as the peak levels of insulin and

fatty acids do not coincide and also, volatile fatty acids are present in only low concentrations in the peripheral circulation. In vitro studies with isolated ovine pancreatic islets, demonstrated that superphysiological concentrations of butyrate, hexanoate and octanoate were more potent than glucose at stimulating insulin release, while acetate, propionate and in addition lactate and 3-OH-butyrate had no stimulating effect (Jordan and Phillips, 1978).

Although infusions of amino acids stimulate insulin the plasma concentrations are considered to be too stable for them to act as regulators of insulin release (Trenkle, 1972).

The gastrointestinal hormones, pancreozymin and secretin, cause a rapid increase in insulin after intravenous infusion, and these may be responsible for the initial increase in insulin after feeding (Trenkle, 1972). Insulin is also released in response to nervous stimulation and stress (Bloom and Edwards, 1975).

Thus the main effects of insulin on metabolism are primarily on the peripheral tissues, and its release in response to a variety of stimuli suggests that it could be important in mediating response to diet, stress and physiological changes.

3.3.2.2 Glucagon:

Glucagon in contrast to insulin, exerts its main effects on the liver.

In vitro studies with sheep hepatocytes have shown a direct effect of glucagon in stimulating gluconeogenesis from propionate (Clark et al., 1976b; Richardson and Livesey, 1979), and, in vivo, glucagon stimulates glycogenolysis and gluconeogenesis

(Brockman and Bergman, 1975a). Normally its effects are modified by a concomitant increase in insulin. However the effects of glucagon alone can be assessed by infusing it into alloxan-treated insulin-infused sheep, where insulin levels remain constant (Brockman and Bergman, 1975a).

Glucagon caused a decrease in plasma amino acids by decreasing non-hepatic utilization and increasing hepatic uptake (Brockman and Bergman, 1975a), and so it appeared to stimulate hepatic gluconeogenesis at the expense of protein synthesis.

A basal level of glucagon is necessary for glucose synthesis as glucagon deficiency (induced by somatostatin) causes a decrease in plasma glucose and glucose turnover (Brockman and Johnson, 1977). It also affects ketone metabolism and when the insulin level was kept constant, glucagon stimulated hepatic ketogenesis and lipolysis, indicated by an increase in plasma glycerol. These changes did not occur however when the insulin levels were allowed to rise normally (Brockman, 1976) and so would not occur in vivo. As glucagon deficiency caused a decrease in plasma 3-OH-butyrate (Brockman and Johnson, 1977) these results suggest that glucagon may be necessary to maintain basal rates of ketogenesis but, in situations of insulin deficiency, fat mobilization and ketogenesis may be stimulated.

Glucagon is released in response to several factors. Levels rise after feeding (Bassett, 1972) and during hypoglycaemia, and decrease in hyperglycaemia (Brockman, 1977). Although glucagon responds to changes in glucose, the levels were not well correlated (Brockman, 1978) and like insulin glucose may not be the most important regulator. The glucagon levels were higher in ewes on roughage diet than grain (Brockman, 1979).

Glucagon tends to be a catabolic hormone which exerts most of its effects on the liver and on hepatic glucose production.

3.3.2.3 The insulin/glucagon ratio:

The insulin/glucagon ratio (I/G) has been suggested to be a more reliable indicator of the carbohydrate status than the absolute concentrations as it reflects the relative balance of the two hormones (Unger, 1971). A low ratio indicates gluconeogenic processes while a high ratio indicates synthetic processes in extra-hepatic tissues. The ratio may be useful in determining the rate of gluconeogenesis from absorbed metabolites (Bassett, 1975).

3.3.2.4 Growth hormone:

Infusion of growth hormone initially caused an insulin-like response with a decrease in plasma glucose and free fatty acids, and this was followed by lipolysis, with increased free fatty acids and hyperglycaemia (Bassett and Wallace, 1966).

Secretion is suppressed on feeding and the plasma concentration is negatively correlated with protein intake (Bassett et al., 1971). Its release is stimulated by some amino acids (Davis, 1972).

3.3.2.5 Catecholamines:

The catecholamines affect fat and carbohydrate metabolism, and their main common effect is to stimulate an increase in plasma free fatty acids through fat mobilization (Bassett, 1970). In addition, adrenaline stimulates glycogenolysis and the resulting hyperglycaemia is heightened by the ability of both adrenaline and noradrenaline to inhibit insulin secretion (Bassett, 1970). By contrast isoprenaline acts as a powerful stimulator of insulin release (Bassett, 1970).

3.3.3 ENZYME REGULATION:

3.3.3.1 Enzyme distribution:

Species differ in the distribution of enzymes within the cell, and as the location of enzymes affects the movement of substrates and products within the cell, membranes may therefore be sites of regulation. In rats, P-enolpyruvate carboxykinase is found mainly in the cytosol, while in ruminants up to 30% is mitochondrial (Taylor et al., 1971; Ballard et al., 1969). In sheep, pyruvate carboxylase is only found in the mitochondria while in cows it is also found in the cytosol (Ballard et al., 1969; Taylor et al., 1971).

3.3.3.2 Hormonal control of enzymes:

Factors which affect the activity of glucose-6-phosphatase regulate the amount of glucose released into the blood. Glucagon caused a slight increase in its activity (Brockman and Manns, 1974).

Fructose-1,6-biphosphatase is affected by diet as its activity is higher in sheep fed grass (where gluconeogenesis is expected to be higher) than in sheep fed concentrates (Pearce and Unsworth, 1976). It is not affected by glucagon (Brockman and Manns, 1974).

Pyruvate carboxylase catalyses the reaction of pyruvate and CO_2 to form oxaloacetate, and is stimulated by short chain acyl compounds (Keech and Utter, 1963). This is likely to be the mechanism by which gluconeogenesis is stimulated after feeding. Its activity is also stimulated by glucagon (Brockman and Manns, 1974), suggesting that glucagon may regulate gluconeogenesis via this enzyme, by determining the amount of pyruvate which goes

to acetyl CoA, for lipogenesis, and the proportion which goes to oxaloacetate for gluconeogenesis. The glucocorticoid cortisone reduces the activity of pyruvate carboxylase, but it does not alter the activity of P-enolpyruvate carboxykinase (Filsell et al., 1969), and it was suggested that the hyperglycaemia produced by glucocorticoids was not directly due to increased gluconeogenesis, but decreased glucose utilization.

P-enolpyruvate carboxykinase activity is not altered by glucagon (Brockman and Manns, 1974) or fasting, but levels are increased in diabetes (Filsell et al., 1969).

As pyruvate kinase causes a 'futile cycle' between P-enolpyruvate and pyruvate it could regulate gluconeogenesis, and the ratio of P-enolpyruvate carboxykinase to pyruvate kinase was suggested as an estimate of the gluconeogenic potential. The ratio increased with the age of the ewe and was highest in pregnancy (Edwards et al., 1975) although figures for lactation were not given.

3.3.3.3 Enzyme changes during pregnancy:

Of the rate limiting gluconeogenic enzymes measured during pregnancy, fructose-1,6-biphosphatase increased tenfold over the last two weeks (Mackie and Campbell, 1972), while the activity of glucose-6-phosphatase did not change. This however did not make it rate limiting as its gluconeogenic capacity was greater than production rates observed in vivo. The rise in fructose-1,6-biphosphatase may have been hormonally mediated to maximize the conversion of substrates to glucose in late pregnancy (Mackie and Campbell, 1972).

3.3.3.4 Enzyme changes in starvation:

Martin et al. (1973) found that glucose-6-phosphatase decreased on fasting and concluded that the response of ruminants to fasting was a decrease in gluconeogenic and lipogenic enzymes. By contrast, Filsell et al. (1969) found that glucose-6-phosphatase, fructose-1,6-biphosphatase and pyruvate carboxylase were all significantly increased in activity, while P-enolpyruvate carboxykinase was not altered. The changes in renal gluconeogenic enzymes were similar to those of the liver (Filsell et al., 1969).

Hence in starved non-pregnant sheep, P-enolpyruvate carboxykinase may limit glucose synthesis, as its activity increases in diabetic sheep (Filsell et al., 1969) consistent with an increased rate of gluconeogenesis.

3.3.3.5 Enzyme changes in the starved pregnant ewe:

Enzyme changes in the fasted pregnant ewe are quite remarkable in the light of current ideas on maternal adaptation to starvation.

While glucose-6-phosphatase levels increased in well fed ewes over the last three weeks of pregnancy, they decreased 17% in underfed ewes (Ford, 1962), and so this enzyme is sensitive to diet reduction.

The activity of pyruvate carboxylase increased in the liver and kidney after five days starvation (Filsell et al., 1969) and is consistent with the theory that glucose production is maximized by a high activity of this enzyme, which ensures that pyruvate is converted to oxaloacetate and glucose, rather than acetyl CoA.

However the activity of pyruvate carboxylase increased more in non-pregnant sheep, than in pregnant sheep where the demand for glucose is considered to be greater (Filsell et al., 1969).

This suggests enzyme activities may limit glucose production in the fasted pregnant ewe compared to the fasted non-pregnant ewe.

P-enolpyruvate carboxykinase levels do not increase in the liver or kidney of non-pregnant ewes, or liver of pregnant ewes on fasting (Filsell et al., 1969). However kidney levels do increase and this suggests that the fasted pregnant ewe has an increased capacity for renal gluconeogenesis.

In diabetes where glucose production is increased, the activities of pyruvate carboxylase, and P-enolpyruvate carboxykinase are much greater (Filsell et al., 1969; Taylor et al., 1971).

Thus, activities of some rate limiting enzymes do not increase on starvation, some increase, but not to the same extent as in the non-pregnant ewe, and most are not as active as in the diabetic sheep. These results suggest that enzyme activities may limit glucose production, and release, in the starved pregnant ewe and therefore contribute to the development of hypoglycaemia.

3.3.4 FEEDBACK MECHANISMS:

The importance of glucose in regulating its own metabolism can be assessed by measuring the effect of an exogenous glucose load. Glucose infusions reduced endogenous glucose production although variability existed between sheep (West and Passey, 1967; Annison and White, 1961). The maximum suppression obtained in fed sheep with infusions of glucose up to 200 g/day was 60%

(Judson and Leng, 1973a). The suppression may have been mediated through insulin, which increases in hyperglycaemia (Boda, 1964), or through actions which decrease the availability of gluconeogenic substrates (Judson and Leng, 1973a).

Thus glucose may partially regulate its own metabolism.

3.3.5 THE ROLE OF OXALOACETATE:

Oxaloacetate is common to the tricarboxylic acid cycle of glycolysis and to gluconeogenesis (Fig. 1).

Ballard et al. (1969) postulated a control mechanism for ruminants based on competition for oxaloacetate, such that gluconeogenesis is favoured when P-enolpyruvate carboxykinase is active, malate dehydrogenase is inactive and NADH is short, while the opposite conditions would favour lipogenesis.

One factor postulated in the aetiology of ketosis was that excess acetyl CoA from fatty acid oxidation was not oxidized, due to oxaloacetate shortage, and so was converted to ketone bodies (see Baird et al., 1974), and some supporting evidence has been found in decreased levels of oxaloacetate in ketosis. However Reid (1968) considered that since oxaloacetate acted catalytically it was not destroyed in the tricarboxylic acid cycle, and supplementation from amino acids should ensure that adequate supplies of oxaloacetate were available. Ballard et al. (1968) found no change in oxaloacetate levels, or in the activities of P-enolpyruvate carboxykinase and pyruvate carboxylase in ketosis and Ford (1963a) found no significant difference in oxaloacetate levels in ketotic pregnant ewes.

This theory, that ketosis is due to oxaloacetate deficiency therefore lacks evidential support.

3.3.6 SUMMARY:

Control of gluconeogenesis is important in understanding what factors maintain homeostasis. If precursors dominate its regulation the question becomes, what happens in starvation, or when precursors are in short supply, as in undernutrition?

In vitro preparations, such as hepatocytes, can be used to study some factors that regulate gluconeogenesis as production rates can be measured in the presence of adequate substrates, and inhibitors and stimulators can be studied independently.

In addition to precursor supply, hormones, enzyme activity and feedback mechanisms all participate in the control of gluconeogenesis.

3.4 UTILIZATION OF GLUCOSE

3.4.1 ENDOGENOUS GLUCOSE:

3.4.1.1 Catabolism:

Glucose has two major functions in the animal. One is a calorific role where it is oxidized to provide energy and the second is a metabolic role, as a substrate for the synthesis of other compounds. In sheep, one-third of the glucose turnover is oxidized and two-thirds used for metabolism (Bergman, 1963).

Glucose cannot be considered to be a major oxidative substrate as it accounts for only 10% of the CO_2 , regardless of the physiological or dietary state (Bergman, 1973). However the effects of hypoglycaemia (Reid, 1951a, b) together with the observations that it is the principal substrate of the brain and is not replaced during

hypoglycaemia or starvation (Kammula, 1976; Lindsay and Setchell, 1976) suggest that glucose has a vital role and is essential for brain metabolism.

Glucose is stored as glycogen in most tissues but particularly the muscle and liver and the quantity stored is affected by diet.

It acts as a reserve and is broken down under conditions such as exercise, hypoxia and acidosis (Scrutton and Utter, 1968).

In a metabolic role glucose is involved in fat synthesis by acting as a substrate for glycerol formation and in providing NADPH. It can also be used for triglyceride synthesis, but in ruminants acetate is the major substrate for lipogenesis (Ballard et al., 1969; Bergman, 1973).

During pregnancy glucose acts as both a fuel and a substrate for the foetus. Uterine uptake of glucose accounted for 70% of the glucose turnover in the ewe (Setchell et al., 1972).

The mammary gland also has a large requirement for glucose that is met in part by increased glucose synthesis from increased food intake (Bergman and Hogue, 1967).

3.4.1.2 Glucose turnover:

If a system is in steady state, the rate a substance enters equals the rate it is lost, and this is called the turnover. In the ruminant little glucose enters from outside via the diet, and so the turnover equals the rate of gluconeogenesis and glycogenolysis. Since at physiological glucose levels none is lost by excretion in the urine, the turnover also equals the rate at which glucose is utilized by tissues.

Various techniques have been used to measure this quantity based on the rate of dilution of radioactive glucose in plasma.

Leng (1970) collated glucose turnover values published by different workers. Measurements for non-pregnant ewes varied five-fold, and he suggested that the variation was due to technique, experimental treatment of the animals and the nutritional regime.

The mode of tracer administration should not affect the results, in theory or in practice (White et al., 1969). However, frequency of feeding and type of diet do have an effect. Large variation occurs in plasma glucose levels of ewes fed once daily (Hodgson and Mellor, 1977). Production and utilization of glucose vary with time after feeding as the availability of gluconeogenic precursors alters. This variability is reduced with more frequent feedings (Hodgson and Mellor, 1977).

The type of diet also affects glucose turnover. Glucose entry rates increase linearly with energy and protein intakes (Judson and Leng, 1968) due to the increased availability of precursors. On rations containing more starch the entry rates of glucose, and production rates of propionate do not change but the proportion of glucose from propionate decreases, suggesting that glucose from the diet is inhibiting glucose synthesis (Judson et al., 1968). Glucose turnover decreases on fasting (Bergman, 1973) and may be related to a lack of precursors.

In addition to diet, glucose turnover varies with physiological state and is increased in pregnancy and lactation (Bergman, 1973).

Many workers have reported a relationship between plasma glucose levels and glucose turnover (Steel and Leng, 1973a; Annison and White, 1961; Bergman, 1973), while others have not found this to be so (Bassett et al., 1971; Judson and Leng,

1973a). As concentrations are functions of utilization and synthesis, simultaneous changes are not necessarily reflected in changed plasma levels, and a steady plasma glucose does not mean that fluctuations in turnover have not occurred. Thus kinetically, plasma glucose and glucose turnover need not be related, but physiologically, they may be.

From the figures presented by Annison and White (1961) and Steel and Leng (1973a) it is doubtful whether the relationship between glucose turnover and plasma glucose was significant during starvation, as glucose turnover was constant over a wide range of plasma glucose values.

The hormonal status of the animal may also influence turnover, and glucose entry rate is positively correlated with insulin concentration (Bassett et al., 1971).

3.4.2 UTILIZATION OF INFUSED GLUCOSE:

The ability of an animal to utilize an injected glucose load is termed its glucose tolerance. In an animal with high tolerance, the load is quickly metabolized and plasma levels promptly return to normal. Concomitant with the glucose disappearance is an initial increase in insulin concentration. The rate of insulin increase has been related to glucose disappearance by an index of sensitivity (Bergman et al., 1979), which is a measure of the sensitivity of tissues to insulin action. The reciprocal is known as the resistance.

Two types of test have been used to assess tolerance. The oral test is considered more physiological in humans (Olefsky et al., 1973) where carbohydrate is absorbed from the digestive tract,

while in the ruminant the intravenous test is the more applicable, as most glucose is synthesized endogenously.

The tolerance test has long been used in human medicine to diagnose diseases which affect glucose homeostasis. These include diabetes (Lundbaek, 1962), gestational diabetes (Silverstone et al., 1961) and renal failure (Horton et al., 1968).

Few tolerance studies have been made in ruminants. On roughage diets sheep had low glucose tolerance but on diets containing maize, disappearance rates were comparable to those in man (Reid, 1958). The hormonal responses were not assessed.

The validity of the tolerance test as an index of glucose metabolism has recently been criticized (Wolfe et al., 1978; Cunningham and Heath, 1978). The K value, which is a measure of the rate of glucose disappearance, was found to depend on factors other than the rate of tissue uptake. These included the amount of glucose injected relative to the glucose pool size, as this affects the diffusion gradient into cells, and the degree to which endogenous production was suppressed, as the rate of disappearance is the difference between uptake and production.

It was shown experimentally that tissues could be taking up glucose at an increased rate, while the K value decreased, and it was concluded that it cannot therefore be considered synonymous with the rate of glucose uptake by tissues (Wolfe et al., 1978).

Cunningham and Heath (1978) investigated tolerance data using mathematical models. The rate of decline was affected by the glucose space, endogenous glucose production and a factor which was a measure of the sensitivity of tissues to insulin.

Wolfe et al. (1978) warned of the dangers in assigning physical definitions to terms used in mathematical descriptions of the data.

3.5 GLUCOSE METABOLISM DURING PREGNANCY

3.5.1 MATERNAL METABOLISM:

3.5.1.1 Plasma metabolites:

Glucose concentration during pregnancy is influenced by nutrition and number of fetuses (Davies et al., 1971). Ewes carrying singles maintained normal blood glucose levels throughout on modest levels of underfeeding, while the same degree of underfeeding significantly reduced the levels in twin-pregnant ewes. Ewes carrying triplets were hypoglycaemic even when adequately fed (Davies et al., 1971).

Plasma glucose increases in all ewes about nine days before parturition (Patterson et al., 1964), and this is considered to be a natural event. Hyperglycaemia has been observed at parturition (Reid, 1960a).

Free fatty acid levels also increase towards the end of pregnancy. This rise cannot be prevented by adequate nutrition, and is possibly the result of hormonal changes (Sykes and Field, 1972; Heaney and Lodge, 1974). Free fatty acids are higher in ewes with multiple fetuses, and are increased in all ewes on undernutrition (Davies et al., 1971).

Ketone body levels of well fed ewes do not change throughout pregnancy (Procos, 1962a). Normal values for acetoacetate are <1 mM, and <2 mM for 3-OH-butyrate (see Lindsay and Leat, 1975).

Ketone bodies are inversely correlated with the calorific intake (Reid, 1968) and underfeeding significantly increased the levels of acetone, which is formed spontaneously from acetoacetate. Acetone levels were also higher throughout pregnancy in ewes carrying multiple foetuses (Davies et al., 1971).

Total plasma protein, albumin and globulin all decreased during pregnancy and this was attributed to a large increase in extracellular fluid during pregnancy, and a protein deficiency of the diet. Further experiments showed that albumin levels were maintained with adequate nutrition, while the decline in globulin levels could not be prevented (Sykes and Field, 1973, 1974; Sykes and Thompson, 1978).

In ewes on adequate diets, amino acid concentrations did not change over the last five weeks of pregnancy, although the ratio of essential to non-essential amino acids decreased during under-nutrition (Slater and Mellor, 1979).

Plasma urea levels are proportional to protein in the diet, and while levels decreased throughout pregnancy on low protein diets, after reaching a minimum at ten weeks on a high protein diet, levels increased to slightly above normal values (Sykes and Field, 1973).

The changes in plasma calcium and phosphorus during pregnancy were small compared to a large 30% change in skeletal composition - plasma calcium fell from 106 to 87 mg/l while phosphorus increased from 48 to 63 mg/l (Sykes and Field, 1974).

3.5.1.2 Glucose turnover:

Glucose turnover increases during pregnancy and this occurs even in ewes on a constant ration, and implies that there is an increase in the inherent ability of the ewe to synthesize glucose (Steel and Leng, 1973a).

A comparison of the regression lines of glucose turnover and plasma glucose concentrations of pregnant, and non-pregnant sheep, showed that the slopes were similar but intercepts differed (Bergman, 1973). Thus for the same plasma glucose concentration, pregnant sheep have a higher glucose turnover. This relationship was considered to explain why Ford (1963b) found the glucose turnover of non-pregnant ewes to be higher than pregnant sheep. The result was attributed to the higher plasma glucose levels (Bergman, 1973).

3.5.1.3 Glucose tolerance:

During pregnancy, in humans, the glucose response curve is similar to the non-pregnant state, but there is a greater release of insulin (Spellacy and Goetz, 1963). The increased levels may be necessary to overcome peripheral resistance to insulin, or factors which antagonize its action, such as thyroxine, glucocorticoids, and growth hormone (Spellacy and Goetz, 1963). Glucagon levels are lower after a glucose load, in pregnancy, and this is attributed to the prolonged hyperinsulinaemia and hyperglycaemia (Daniel et al., 1974).

The ability to synthesize and secrete insulin increases in pregnancy in rats (Bone and Howell, 1977). However in humans

insulin deficiencies are commonly seen when the increased requirement is not met, and this condition is called 'gestational diabetes'.

Alterations in carbohydrate and insulin metabolism are also observed in the pregnant ruminant. Insulin levels decline in late pregnancy and smaller responses are seen after feeding (Hove and Blom, 1976). By contrast, growth hormone levels increase, and the responses to feeding are more pronounced in late pregnancy, particularly in multiple pregnant sheep (Hove and Blom, 1976). This is thought to assist glucose homeostasis, by reducing glucose consumption, and increasing lipolysis.

Thus, while glucose tolerance is normal during pregnancy, there is an increase in insulin resistance, and so larger amounts of insulin are required to attain the same rates of glucose uptake.

3.5.1.4 Hormonal changes:

During the last five weeks of gestation plasma insulin levels decrease and the reduction is largest in underfed, multiple pregnant ewes (Blom et al., 1976; Hove and Blom, 1976). The decrease in insulin is correlated with a decrease in glucose. Glucagon levels have not been monitored throughout pregnancy.

Growth hormone levels increased, from 3 to 12 ng/ml in late gestation, and, as this increase was correlated with increases in free fatty acids and number of foetuses, the stimulus was suggested to be the increase in foetal nutrient requirement (Blom et al., 1976; Hove and Blom, 1976). Consistent with this theory were the observations that the increase occurred earlier in underfed ewes, and tended to reach higher levels in multiple pregnant ewes (Blom et al., 1976).

Ovine placental lactogen (OPL) is produced by the placenta and is detectable in maternal plasma from day 40 of gestation. Levels peak between 100-130 days, and then decrease (Handwerger et al., 1977; Kelly et al., 1974; Taylor et al., 1978). The levels were affected by number of fetuses, and were higher in ewes with twins (Handwerger et al., 1977).

While the liver has the highest specific binding of OPL (Chan et al., 1978) its effects are widespread. Injections caused prolonged decreases in free fatty acids, glucose and amino nitrogen, while insulin decreased initially and then increased (Handwerger et al., 1976). It is considered to be important in the regulation of intermediary metabolism during pregnancy.

Thyroxine levels were stable over the last five weeks of pregnancy, even during underfeeding (Mellor et al., 1977a).

There was no increase in cortisol in sheep, due to pregnancy per se (Reid, 1968) and maternal corticosteroids remained relatively stable (5-15ng/ml) over the last three weeks of pregnancy (Mellor et al., 1977b).

In summary, hormonal changes during pregnancy are generally small but the changes are amplified by undernutrition and multiple pregnancy.

3.5.2 FOETAL METABOLISM:

The foetus is dependent upon the ewe for substrate supply and end-product removal. Although some aspects of foetal metabolism, such as substrate uptake in the fed ewe, have been well researched, other areas such as the gluconeogenic capacity of the foetus in vivo, and its ability to regulate its own metabolism, are still under consideration.

Prior to the development of techniques for chronic foetal catheterization a problem existed in measuring substrate uptake, as uterine uptake measured uptake and metabolism of the uterus, placenta and foetus. It is now possible to study the metabolism of each organ separately.

3.5.2.1 Glucose and fructose:

Fructose is a mystery substrate, which is synthesized from glucose by the placenta, and its concentration in the foetus varies from 102 mg/dl in mid-gestation to 58 mg/dl in late gestation (Hitchcock, 1949). Despite these high concentrations it is only slowly metabolized and accounts for 3% CO₂ produced by the foetus (Alexander et al., 1969). Warnes et al. (1977a) concluded that as fructose was oxidized slowly, was not converted to glucose, lactate, glycogen or lipids, and did not pass through the placenta, it was unlikely to be important as an energy reserve in the foetus.

Consistent with the low rates of metabolism, no umbilical arterio-venous differences were recorded (Hitchcock, 1949) and this was so even in fasted sheep (Tsoulos et al., 1971). Fructose is not considered to be a major substrate or regulator of foetal metabolism.

By contrast, glucose is taken up and readily metabolized by the foetus, although the blood concentrations are low, at 10-30 mg/dl (Hopkins, 1975). Glucose metabolism accounts for 50% of oxygen taken up by the foetus (Tsoulos et al., 1971) and it is incorporated into glycogen, lactate, fructose and is oxidized to CO₂ (Warnes et al., 1977a; Setchell et al., 1972).

Uterine glucose uptake was 48 mg/min while foetal uptake was 17 mg/min and so only a fraction (35%) of the glucose taken up by the uterus reached the foetus (Simmons et al., 1975).

Foetal glucose uptake is correlated with maternal arterial glucose concentration, and also maternal arterial-foetal umbilical concentration difference (James et al., 1972), so factors which alter these concentrations affect glucose uptake. Increasing maternal glucose concentration increased foetal glucose uptake, and it decreased when the ewe became hypoglycaemic (Battaglia and Meschia, 1973; Prior and Christenson, 1978a). Infusion of insulin reduced maternal concentrations by 50% (64 to 31 mg/dl) while uterine uptake fell by 75% (21 to 5 mg/kg foetus/min) (Prior and Christenson, 1978a). Uptake was increased when foetal glucose concentrations were lowered (Simmons et al., 1978).

Other factors apart from glucose concentration differences also affect glucose uptake by the pregnant uterus. Insulin seems to be able to directly affect the permeability of the placenta to glucose, as infusions at rates which did not affect maternal glucose concentrations, caused a 45% increase in glucose uptake (Paxson et al., 1978).

Total uterine uptake increases with the stage of gestation and with the number of fetuses, but uterine uptake per kg foetal weight was constant throughout pregnancy at 17 mg/min/kg (Christenson and Prior, 1978).

3.5.2.1.1 Mechanism of glucose uptake:

Glucose crosses the placenta by facilitated diffusion and insulin is considered to affect transport by either increasing the carrier

numbers or affinity, up to a maximum, when all receptor sites are saturated (Paxson et al., 1978).

3. 5. 2. 1. 2 Regulation of glucose metabolism in the foetus:

There is considerable evidence that the foetus is able to regulate its own glucose homeostasis in the last third of pregnancy. Insulin and glucagon are both present in the foetal pancreas from day 50, insulin release is stimulated by glucose and inhibited by adrenaline, and glucagon release is stimulated in the foetus with alanine and adrenaline (Bassett, 1977), and so both hormones are probably secreted in vivo by the foetal lamb.

Insulin directly affects foetal glucose uptake and utilization (Simmons et al., 1978) and while the role of glucagon is not certain, it may be involved in mobilization of foetal glycogen (Bassett, 1977).

One situation where regulation by the foetus is seen is during maternal hypoglycaemia of starvation. It was postulated that the foetus may adapt by decreasing its insulin concentration, and hence glucose uptake (Bassett, 1977). This was confirmed by Phillips et al. (1978) who observed that foetal plasma insulin decreased from 10 to 3 μ U/ml after 3 days of fasting.

3. 5. 2. 1. 3 Gluconeogenesis and the foetus:

The foetal lamb possesses all the enzymes necessary for glucose synthesis, by late gestation (Stevenson et al., 1976). Of the four potential rate limiting enzymes, P-enolpyruvate carboxykinase is active throughout gestation at a level greater than in the adult, and glucose-6-phosphatase, fructose-1,6-biphosphatase and pyruvate carboxylase increase in a surge from 100 days onwards (Stevenson et al., 1976; Warnes et al., 1977b). Two enzymes necessary for

the synthesis of glucose from amino acids, aspartate- and alanine-transaminases, are also active from day 45 (Stevenson et al., 1976). This suggests that from day 100 the foetal liver is capable of synthesizing glucose.

Gluconeogenic enzymes are also present in the foetal kidney. They develop in a similar pattern to the liver, and as in the adult are the more active (Stevenson et al., 1976).

Studies with liver slices showed that the four-month foetal liver can perform gluconeogenesis by converting pyruvate to glucose (Ballard and Oliver, 1965) and the rates were comparable to those of the adult (Ballard et al., 1969). Similar results were obtained in vivo by Prior and Christenson (1977) who found that [U-¹⁴C]alanine was incorporated into glucose by the foetus and alanine contributed 2% to the glucose turnover of 44 g/day. However this figure may have overestimated glucose turnover, as tracer is lost across the placenta.

Conflicting data has been presented by Warnes et al. (1977b). They found that lactate was not incorporated into glucose prior to birth, although an active pathway was present within two minutes of birth. The pathway could not be induced with adrenaline or glucagon before birth, and they postulated that gluconeogenesis by the young ruminant may be initiated by the increased oxygenation of the blood, which altered the redox state of some coenzymes.

3.5.2.1.4 Glycogen reserves:

From day 60 foetal lambs accumulate glycogen in various tissues, and by the end of gestation the concentrations in liver (72 mg/g), heart (14 mg/g) and muscle (42 mg/g), were up to five times the adult level (Setchell et al., 1972).

The stores are rapidly broken down at birth (after two hours only 10% remain) and by anoxia, and are considered to assist in temperature regulation (Shelley, 1961). As maternal nutrition may affect glycogen storage, it may ultimately affect the lamb's survival (Shelley, 1961).

3. 5. 2. 2 Other substrates:

Glycerol and free fatty acids are taken up in small quantities by the placenta but account for only a fraction of the CO₂ produced (Battaglia and Meschia, 1973; Alexander et al., 1969). The uptakes are not increased during starvation (James et al., 1971) and so they are not considered to be major metabolic fuels.

Ketone bodies are metabolized slowly within the foetus, and as the placenta is relatively impermeable to them, they also do not contribute significantly to foetal metabolism (Alexander et al., 1969).

Since glucose metabolism can only account for 50% of the oxygen uptake, it was postulated that amino acids and urea metabolism may be important. It was found that 25% of the uptake could be accounted for by catabolism of amino acids, determined by urea production (Gresham et al., 1972). The placenta takes up amino acids at a rate independent of the maternal concentration, and against a concentration gradient (Young and McFadyen, 1973). It was considered that some transamination and oxidation occurred within the foetus, as some amino acids were taken up surplus to requirements and glutamate was actually secreted (Lemons et al., 1976).

The placenta is relatively impermeable to lactate and so it is not taken up from the maternal circulation, but is synthesized by

the placenta. The amount released into the foetal circulation is sufficient to account for 25% of oxygen uptake (Burd et al., 1975).

Acetate, the predominant fatty acid in the maternal circulation, is not taken up or appreciably metabolized by the foetus (Scarlsbrick and Pugh, 1957).

3.5.3 PLACENTAL METABOLISM:

The placenta is a barrier through which all substrates moving between the mother and foetus must pass. It is also an organ capable of metabolism, and several functions have been defined.

The absence of glucose-6-phosphatase (Stevenson et al., 1976) and P-enolpyruvate carboxykinase (Edwards et al., 1977) indicated that the placenta was incapable of gluconeogenesis. However, the high activities of pyruvate kinase indicated a high rate of glucose catabolism by glycolysis (Dhand et al., 1970; Edwards et al., 1977).

In vivo, the uterus took up glucose at 48 mg/min but glucose crossed to the foetus at only 17 mg/min, and so 65% of the glucose (31 mg/min) taken up appeared to be metabolized by the placenta (Simmons et al., 1975). The glucose was rapidly converted to fructose which was released into the foetal circulation (Britton et al., 1967) and lactate (Burd et al., 1975) which was released into the maternal and foetal circulations (Edwards et al., 1977).

Pyruvate was released into the maternal circulation (Burd et al., 1975) and may have been synthesized in the placenta from alanine (Edwards et al., 1977). From enzyme studies it was concluded that the placenta was able to oxidize ketone bodies, but was unlikely to produce urea (Edwards et al., 1977).

Another function of the placenta is hormone synthesis and in late pregnancy, it releases ovine placental lactogen (OPL) and progesterone (Kelly et al., 1974).

3.6 GLUCOSE METABOLISM DURING STARVATION

Since food is retained in the rumen for 48 hours after feeding (Reid, 1968) only periods greater than two days will be considered as starvation.

3.6.1 NON-PREGNANT EWES:

3.6.1.1 Plasma metabolite changes:

During starvation volatile fatty acid production in the rumen ceases and, as plasma acetate falls from 25 to 2 mg/dl (Reid, 1968), other metabolites must act as oxidative substrates. These metabolites are obtained by mobilization of body reserves, and during fasting 75% of the heat produced is from oxidation of body fat and 25% from body protein (Graham, 1964).

Mobilization of fat produces free fatty acids and glycerol. Fatty acids increased rapidly on fasting to ten times the levels of fed ewes (Annison, 1960), and could thus be important substrates. They are oxidized to acetyl CoA by peripheral and hepatic tissue and the acetyl CoA so formed can either enter the tricarboxylic acid cycle or be converted to ketone bodies by the liver (see Reid, 1968).

Increases in glycerol were more modest - from 35 to 93 μ M in ewes fasted 3-5 days (Bergman et al., 1968), and it contributed more to glucose production - 5% of glucose turnover in the fed state and 23% in fasting.

Ketone bodies are produced by the liver and kidney but are utilized only by extrahepatic tissues (Kaufman and Bergman, 1974). Levels increased threefold on starvation, from 2 to 6 mg/dl (Procos, 1962b; Varnam et al., 1978). Acetoacetate, the parent ketone

body, increased throughout starvation while 3-OH-butyrate reached a maximum after four days and then decreased (Procos, 1962b). Ketones are considered to be important oxidative substrates in starvation (Reid, 1968) and in vivo, renal ketone utilization increased during fasting (Kaufman and Bergman, 1971). However, conflicting evidence was obtained in vitro as the activities of enzymes required for ketone body oxidation decreased in the heart, brain and kidney during fasting (Varnam et al., 1978) suggesting that the ability of the animal to use ketone bodies is lower during starvation.

Glucose levels alter to varying degrees. In ewes fasted for six days levels decreased 50% (Varnam et al., 1978) but prolonged fasting of wethers for 27 days produced no change (Martin et al., 1973). These conflicting results may be explained by the work of Procos (1962b). He noted that the change in glucose during fasting was related to the diet previously fed. Sheep on high protein diets had low blood glucose levels which showed little change on fasting, while those on high carbohydrate diets had high levels which were greatly reduced on fasting (Procos, 1962b). Earlier studies in rats had shown that animals on high protein diets had higher fasting glucose and glycogen levels, which suggested that those on protein diets had higher rates of glucose synthesis (Mirski et al., 1938). The protein effect disappeared when the adrenals were removed (Mirski et al., 1938) implicating adrenal hormones as the mediators of this effect.

Insulin levels were positively correlated to protein intake, and the amount of protein digested in the intestine may be the main dietary factor regulating insulin concentrations in sheep (Bassett

et al., 1971). This observation would link carbohydrate metabolism to protein metabolism.

Fasting causes protein mobilization, and whereas the muscle takes up amino acids in the fed situation, during fasting it releases them, with the greatest output of glycine and alanine, at 4-5 $\mu\text{mol/kg muscle/min}$ (Lindsay et al., 1977). Arterial concentrations, however, are reduced and this is possibly due to increased uptake by the liver and kidneys (Lindsay et al., 1977; Bergman et al., 1974). Urea levels, which reflect protein catabolism, were reduced in ewes fasted six days (Herriman and Heitzman, 1978).

The plasma changes in non-pregnant ewes on starvation are therefore a mild ketosis and increased free fatty acids. The changes in some metabolites are related to previous feed composition. In particular the response of glucose is related to protein content of the diet.

3. 6. 1. 2 Glucose turnover:

Even though plasma glucose levels may not alter significantly on fasting, great changes occur in glucose turnover. In sheep fasted for four days an 8% decrease in plasma glucose was accompanied by a 35% decrease in turnover (Bergman, 1973). Glucose decreased from 59 to 54 mg/dl, and turnover from 2.08 to 1.36 g/kg/day.

Changes are rapid, and a 44% decrease in turnover was recorded in sheep fasted two days (Annison and White, 1961), but there was evidence that a basal rate was attained after four days. Steel and Leng (1973a) recorded a 36% reduction in irreversible loss after two days starvation (from 2.26 to 1.44 g/kg/day), 51% after four

days (to 1.11 g/kg/day), but no further decrease by six days (1.15 g/kg/day). The plasma glucose concentrations showed little change over the six days and makes the correlation between glucose turnover and plasma glucose levels doubtful during starvation.

The results of Steel and Leng (1973a) suggested that recycling may have increased in starvation, but Brockman et al. (1975b) using the double isotope technique, established that recycling was about 5% in feeding and fasting.

3.6.1.3 Hormonal changes:

No change was recorded in blood glucose or insulin after a seven day fast (Boda, 1964) but when glucose was reduced, plasma insulin also decreased (Bassett, 1972; Horino et al., 1968; Trenkle, 1976). The levels of insulin decreased after one day from 35 to 12 μ U/ml and to a minimum at three days of 4 μ U/ml and no further change occurred up to six days of fasting (Horino et al., 1968). In vitro studies have shown that insulin synthesis and secretion by the rat pancreas is reduced in starvation (Bone and Howell, 1977).

Plasma glucagon decreased steadily over four days starvation, but to a smaller extent than insulin, so the I/G ratio decreased favouring gluconeogenesis (Bassett, 1972).

Growth hormone levels increased slightly (Trenkle, 1976) or remained stable (Bassett, 1972; Machlin et al., 1968) and would thus not seem important in regulation of glucose metabolism during starvation. Similarly, cortisol remained at normal levels (0.6 μ g/dl, Reid, 1968) after a six day fast (Bassett et al., 1966).

Similar changes in insulin, growth hormone and corticoids were observed in fasted cows, but it was noted that the hormonal changes seemed to follow rather than initiate plasma metabolite changes (Athanasίου and Phillips, 1978).

Studies on hormone kinetics may be necessary to define their roles in adaptation to starvation, but insulin and glucagon levels decrease and growth hormone and cortisol levels are generally unchanged.

3.6.2 PREGNANT EWES:

3.6.2.1 Plasma metabolite changes:

Some of the changes induced by starvation in the pregnant ewe are similar to those seen in non-pregnant sheep, such as decreased volatile fatty acids and glucose, and increased free fatty acids and ketones. However, in addition, HCO_3^- decreases and urea and creatinine levels rise, due to decreased clearance of these compounds (Reid, 1968).

The earliest changes that occur in partial and complete starvation are a prompt fall in glucose and increase in free fatty acids, and these are obvious 24 hours after feeding (Bassett and Madill, 1974). Hypoglycaemia occurs irrespective of previous nutrition, or stage of pregnancy (Steel and Leng, 1973a). The glucose levels appeared to reach a minimum after two days and were not altered further, for up to 10 days (Simmons et al., 1974).

Free fatty acid levels doubled after three days starvation and their uptake by the liver increased (Katz and Bergman, 1969b). The changes were smaller than those observed in fasted, non-pregnant ewes; however a larger proportion were converted to

ketone bodies (Katz and Bergman, 1969b). In the fed animal ketone bodies are produced in the portal bed from butyrate, but during fasting they are produced by the liver from free fatty acids. The plasma ketone levels increased to a larger extent in pregnant ewes due to the increased production (Katz and Bergman, 1969b). The turnover (and hence utilization) increased to a maximum at 20 mg/dl (or 10 mg/dl acetoacetate) when further production resulted in large increases in plasma levels (Bergman and Kon, 1964). Renal utilization of ketone bodies does not increase; however a larger proportion of their uptake is excreted (Kaufman and Bergman, 1974).

Glycerol is released along with free fatty acids, from adipose tissue, and the levels increased threefold on fasting (Kaufman and Bergman, 1974). It was considered that this increase may indicate that glycerol becomes a major glucose precursor during starvation along with lactate and amino acids. However, lactate levels do not change on starvation (Kaufman and Bergman, 1974; Herriman and Heitzman, 1978).

Amino acid concentrations decrease on starvation of the pregnant ewe, and this is attributed to increased hepatic uptake (Bergman et al., 1974). Urea levels, which reflect amino acid catabolism increased from 40 to a peak of 80 mg/dl after two days of starvation, and then declined to levels slightly above normal (Simmons et al., 1974). This rise and fall could explain why Herriman and Heitzman (1978) found no change in urea levels in ewes starved for six days.

Cation composition of the plasma alters during starvation with decreases in K^+ , Ca^{++} and Mg^{++} although Na^+ concentrations do not change (Wolff et al., 1974).

3.6.2.2 Liver composition:

Underfeeding pregnant ewes over the last three weeks of gestation decreased the water content of the liver from 72% to 65% (Ford, 1962). It also decreased the glycogen content, from 4.7 to 1.2% wet weight, although part of this reduction could be attributed to pregnancy per se as levels decreased (4.7 to 3.6% wet weight) even in adequately fed ewes (Ford, 1962).

The fat content remained stable in adequately fed ewes, at 4% wet weight, at the end of gestation, although increases were detected histologically (Ford, 1962; Patterson et al., 1964). Large increases in fat content, however, were noted in underfed ewes and levels increased to 19% wet weight (Ford, 1962).

Thus the alterations in hepatic composition in late pregnancy may reflect altered hepatic metabolism, and these changes were most severe during undernutrition.

3.6.2.3 Glucose turnover:

Glucose turnover rates decrease on starvation in the pregnant ewe (Kronfeld and Simesen, 1961; Leng, 1970; Bergman, 1963; Ford, 1963b; Katz and Bergman, 1969b; Annison et al., 1967; Steel and Leng, 1968, 1973a).

A summary:

TABLE 1: Glucose Turnover in Pregnant Ewes.

Starvation Period (days)	Glucose Turnover (g/kg/day)		Decrease %	Reference
	Fed	Starved		
-	3.26	1.23	62	Kronfeld & Simeson, 1961
3-6	2.33	1.53	34	Bergman, 1963
4	1.95	1.76	10	Ford, 1963b
4	3.39	1.63	52	Steel and Leng, 1968
3	3.66	1.64	55	Katz and Bergman, 1969b
1	2.99	2.24	25	Annison <u>et al.</u> , 1967

The turnover rate reflects the rate of utilization and synthesis, and the question becomes why are they reduced during starvation? Synthesis could be impaired by lack of precursors, and utilization by altered hormonal status, such as decreased insulin.

Since propionate is not absorbed during starvation, other gluconeogenic precursors become important, and these are glycerol, lactate and amino acids (Bergman, 1973).

Glycerol levels increase in starvation, and the proportion of glucose from glycerol increased from 5% in fed non-pregnant ewes to 28% in hypoglycaemic pregnant ewes. However, even if all the glycerol released was converted to glucose in the starved ewe, it would only provide 55% of glucose requirements (Bergman et al., 1968).

Lactate levels did not increase in starvation (Kaufman and Bergman, 1974) and as glucose formation from lactate is a function of lactate concentration (Reilly and Chandrasena, 1978) this suggests that there is no increase in lactate conversion to glucose. Lactate

contribution to renal gluconeogenesis actually decreased during starvation (Kaufman and Bergman, 1974).

The contribution of amino acids to glucose in the starved pregnant ewe has not been directly assessed.

There was no difference in glucose turnover of starved ewes in mid- and late-gestation (Steel and Leng, 1973a) which suggested that, as pregnancy progressed, there was no change in the ability of a ewe to synthesize glucose from her body reserves.

Utilization of glucose is not considered to change during pregnancy and this conclusion is based on oxidation rates. Production of CO_2 , which is a measure of metabolic rate, decreased on fasting. However the proportion of glucose which was oxidized (30%) and the proportion of CO_2 from glucose (10%) were constant in fed and fasted sheep (Bergman, 1963), and suggested that there was no shift away from glucose as a catabolic fuel.

Bergman (1963) suggested that hypoglycaemia of the underfed ewe was therefore due to insufficient glucose production.

3.6.2.4 Renal gluconeogenesis:

The kidney is capable of gluconeogenesis and ammoniogenesis and differs from the liver in that its membrane is permeable to tricarboxylic acid cycle intermediates and so these are capable of stimulating gluconeogenesis (Pogson et al., 1976).

In rats renal gluconeogenesis increased in starvation and acidosis (see Pogson et al., 1976), but early studies indicated that this was unlikely to occur in ruminants as nearly all glucose turnover could be accounted for by liver production (Bergman et al., 1970). This has been confirmed by subsequent in vivo and in vitro work.

When net renal production was measured, glucose synthesis increased in pregnancy but not in fasting. However, renal production accounted for a larger proportion of glucose turnover (15%) in the fasted ewe, than the fed ewe (10%) (Kaufman and Bergman, 1974).

Lactate was the predominant substrate and accounted for about 50% of the renal glucose produced, while glycerol and pyruvate together accounted for 20% (Kaufman and Bergman, 1974). Amino acids probably accounted for the rest as alanine uptake by the kidneys continued in starvation, glutamine (which is normally released) was also taken up, and glutamate, a product of glutamine catabolism, was released (Bergman et al., 1974). Thus there appeared to be considerable renal metabolism of amino acids, and these may act as links between glucose synthesis and excretion. Deamination of glutamine releases NH_3 , which buffers acid in the urine and conserves Na^+ , and the carbon skeleton glutamate which can be synthesized into glucose (see Bergman et al., 1974).

The role of amino acids was also demonstrated in sheep kidney cortex slice experiments where, after two weeks starvation, gluconeogenesis from most substrates decreased. However rates were maintained or increased with glutamate, serine and alanine (Sasaki et al., 1975).

Renal glucose production was increased in acidotic sheep from 2 to 6 g/day, which is only 6% of glucose turnover (McIntosh et al., 1973).

Hence renal gluconeogenesis does occur in sheep, but under the conditions of feeding, fasting, pregnancy and acidosis, it only accounts for 15% of glucose turnover and so makes a relatively small contribution to glucose metabolism.

3.6.2.5 Hormonal changes:

The studies on hormonal changes on maternal fasting are limited to date.

Plasma insulin decreased on two days fasting in late pregnancy from 20-25 to 6 μ U/ml but, in contrast to the non-pregnant ewe (where levels decreased) glucagon levels did not change (Bassett and Madill, 1974; Bassett, 1977). This decreased the insulin/glucagon ratio to a greater extent in the fasted pregnant ewe, favouring gluconeogenesis.

While no change was observed in growth hormone or corticosteroid levels in the non-pregnant ewes, fasting pregnant ewes for two days increased growth hormone levels (from 3 to 8 ng/ml) and corticosteroid levels (from 8 to 15 ng/ml) (Bassett and Madill, 1974).

Thyroxine levels remained stable during seven days underfeeding in late pregnancy (Mellor et al., 1977a).

From published data the time course of metabolite changes relative to hormone changes cannot be determined. However, there was evidence that new plateau levels had been reached after 24 hours starvation (Bassett and Madill, 1974).

Assessment of hormonal changes are required for periods of starvation greater than two days as, after this time, precursors from the rumen would not be available, tissue mobilization would begin, and other adaptations to maintain homeostasis may be apparent.

3.6.2.6 Foetal response to maternal starvation:

During starvation maternal and foetal glucose concentrations fall (Tsoulos et al., 1971; Boyd et al., 1973; Bassett and Madill,

1974; Simmons et al., 1974; Mellor et al., 1977a). Maternal plasma glucose decreased from 80 to 30 mg/dl and foetal levels from 26 to 17 mg/dl after two days of starvation and both remained at these levels for up to 10 days of starvation (Simmons et al., 1974). This decreased the glucose gradient between maternal and foetal circulations and foetal uptake decreased to 1.5 mg/min/kg, which was one third of normal (Simmons et al., 1974). Despite the fall in glucose uptake, oxygen uptake did not change (Boyd et al., 1973) and while glucose oxidation accounted for 50% of oxygen uptake during feeding, during fasting it only accounted for 17% of the uptake (Tsoulos et al., 1971). Other substrates therefore increase in importance as metabolic fuels for the foetus during starvation.

Studies to determine the contribution of amino acids in maternal starvation were undertaken by Simmons et al. (1974). Urea differences between the foetus and mother peaked after four days and implied that the initial foetal response was increased amino acid catabolism. However, after four days the difference in urea levels decreased and while glucose uptake remained low, foetal glucose levels were constant (Simmons et al., 1974). The substrates which are therefore used to maintain foetal glucose homeostasis after four days of starvation are unknown.

Foetal hormones are considered to play a role in foetal adaptation to maternal starvation. Foetal insulin decreased and growth hormone increased after two days of fasting (Bassett and Madill, 1974), and in prolonged starvation foetal corticosteroids increased, although unlike insulin and growth hormone, these can cross the placenta from the mother (Mellor et al., 1977a). Foetal glucocorticoid production may also have occurred, as hypoglycaemia

increased foetal ACTH and this may have directly stimulated secretion from the foetal adrenal (Jones, 1976). These changes would have tended to reduce glucose utilization in the foetus. Thus the foetus appears to respond to maternal starvation by reducing its requirements for glucose, and utilizing other, as yet unknown, substrates. That foetal metabolism is decreased is shown by the lower birthweights of lambs from undernourished ewes (Robinson, 1977).

3.7 LOSS OF HOMEOSTASIS; HYPER- AND HYPOGLYCAEMIA.

3.7.1 ROLE OF THE LIVER:

The liver, for several reasons, can be considered to play a vital role in glucose homeostasis. As outlined in earlier sections, in ruminants it is the main producer of glucose. In addition, it is exposed to higher insulin concentrations than peripheral tissues and is the main site of insulin extraction (Brockman and Bergman, 1975b). Finally, it appears to be the prime target of glucagon action (Brockman et al., 1975a).

As testimony to its role, liver dysfunction has been implicated in many carbohydrate disorders such as diabetes and obesity (Felig and Sherwin, 1976).

3.7.2 HYPOGLYCAEMIA: (The effect of insulin infusion)

Ruminants have a high tolerance to severe hypoglycaemia, and when insulin doses are increased, the duration rather than depth of hypoglycaemia is increased (Reid, 1951a). Part of this tolerance may be due to increased extraction of insulin by the liver, which occurs during hypoglycaemia (Brockman and Bergman, 1975b).

This prevents insulin reaching the systemic circulation and promoting further glucose uptake.

Glucagon is released in response to insulin-induced hypoglycaemia (Brockman, 1977) and, when it was infused into hypoglycaemic ewes starved for ten days, it increased glucose concentration (Burtis et al., 1968). The increase was considered to be due to gluconeogenesis, as glycogen levels would be low, and subsequently it has been shown that glucagon directly stimulates the gluconeogenic enzyme pyruvate carboxylase (Brockman and Manns, 1974).

The increase in glucagon in hypoglycaemia is followed by an increase in insulin which stimulates uptake of the glucose produced, and normal glucose levels are restored. Other hormone systems may be involved to promote tissue mobilization and provide glucose precursors.

A failure to correct hypoglycaemia could be due to a defect in one or several mechanisms in this response.

3.7.3 HYPERGLYCAEMIA: (Diabetes, glucose infusion)

Normally infusions of glucose cause insulin levels to increase. The insulin stimulates glucose uptake and inhibits endogenous glucose production until the glucose level returns to normal.

If hyperglycaemia was prolonged experimentally, by using alloxan to destroy Beta cells in the pancreas, increases occurred in acetate, free fatty acids and 3-OH-butyrate (Jarrett et al., 1974) indicating that alterations in fat and carbohydrate metabolism had occurred. Appetite was also depressed.

The failure to correct hyperglycaemia could be due to a defect in the insulin system - either the secretion or degradation

of insulin, or a resistance of the liver or tissues, to the action of insulin.

3.8 GLUCOSE KINETICS

3.8.1 KINETIC THEORY AND MODELLING:

Kinetics is the study of movement, or rate of change with time, and tracer kinetics is the study of movement of a compound by using radioactive tracers as labels. Assuming the tracer does not behave differently from the unlabelled substance (tracee), the movement of tracee can be inferred from movement of tracer.

Tracer kinetics has a particular application in animal studies for measuring production, utilization and movement of a specific compound. Hence, if radioactive glucose ($[^{14}\text{C}]$ or $[^3\text{H}]$ glucose) is injected into the bloodstream the loss of activity can be monitored in succeeding blood samples. The tracer leaves the system (animal) as $^{14}\text{CO}_2$ or $^3\text{H}_2\text{O}$ or by conversion to products which are excreted or stored in tissues.

The rate of disappearance of tracer from the plasma can be represented by a set of mathematical equations, called a model.

If the substance is localized in discrete states (which may be physical or chemical) and the rate of loss is proportional to the concentration, the system can be described by a set of differential equations, and is called a compartmental model.

The theory of kinetic analysis and compartmental modelling has been described by Berman et al. (1962a, b) and Berman (1965).

3.8.1.1 Devising a model:

The proposed model is normally based on the number of exponentials required to fit the data. If n exponentials are required, then n compartments are included in the model (Berman, 1962b).

The interconnections between compartments are based on knowledge of the system, but are constrained by the number of compartments sampled. For an n compartment model, sampling one compartment allows only $2n-1$ rate constants to be uniquely determined (Berman and Schoenfeld, 1956).

3.8.1.2 Fitting the model to the data:

The model can be fitted with a suitable computer program. One currently in wide use is SAAM (Simulation, Analysis and Modelling) (Berman and Weiss, 1974). Features and use of the program were described by Berman (1965b), and the notation after Brownell et al. (1968) is summarized in Table 2.

Once a structure, or model, is proposed for the system, the set of equations together with observed data, initial conditions and estimates of the parameters are submitted to the program. It then determines the parameter values and coefficients by statistical fitting to the experimental data, to give a least squares solution (Berman, 1962a). Values are calculated for each observed data point.

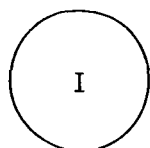
3.8.1.3 Goodness of fit:

The validity of the model is assessed by how well the predicted, fit the observed values. This can be determined by visually appraising the graphs of the two sets of values. Systematic deviations imply that the model is insufficient to explain the data. Another criterion for assessing the fit is by the precision of the parameters. Well determined parameters have low variances and mean a good fit of predicted to observed data (Berman, 1969).

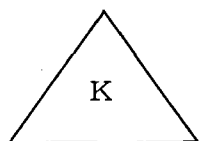
TABLE 2: SAAM Notation.

SYMBOL:

1. Mathematical definition
2. Physical definition



1. A function $F(I, T)$ where I is a component number and T is an independent variable, e. g. time
2. A compartment or its contents, e. g. tracer



1. A linear combination of functions (a summer)
2. The sum of parts of, or more than one compartment

IC(I)

1. Initial conditions for the solution of a set of differential equations
2. Material in a compartment at the start of solution, e. g. injected tracer dose

TC(N)

1. A signal to interrupt the solution to change initial conditions, parameter values or steady state variables
2. Changes during the experiment, e. g. injection of a second tracer dose

L(J, I)

1. Probability that a particle will move from I to J per unit time
2. Fraction of material transported from compartment I to J per unit time, and is called a fractional flow rate

TABLE 2 (Continued)

S(K, I)

1. A summing coefficient
2. The fraction of compartment I that is 'seen' in compartment K

K(I)

1. A proportionality coefficient
2. The fraction of compartment I that is 'seen' in a given amount of sample

P(N)

1. A parameter not associated with a compartment
2. May or may not have physical meaning

M(I)

1. The steady state solution for compartment I
2. The mass of tracee in compartment I

R(J, I)

1. The steady state flux from compartment I to J per unit of time
2. The transport of tracee from compartment I to J per unit of time

U(I)

1. The steady state input into compartment I
2. The entry of tracee into compartment I from the outside

QC(J, T)

1. A function calculated from the $F(I, T)$ and parameters
2. The value of a calculated quantity obtained during the solution of a model

QO(J, T)

1. The observed value of the dependent variable
2. The quantity observed, or measured, e.g. specific activity.

Even if the model fits the data well, it must still be considered a theory, and not a proof (Garfinkel, 1969), and experimental validation is required.

If the initial model does not fit, different configurations have to be considered.

3.8.1.4 Developing the model:

Once a model has been developed to explain one set of data, it is then necessary to explore how well it describes the system in other situations. To do this, more experiments are necessary, first after perturbing the system to a new steady state, and then by non-steady state analyses (Berman, 1969).

The ultimate aim is to understand the system, by developing a unique model which will predict its behaviour under all circumstances.

3.8.2 TRACER EXPERIMENTS:

The type of experiment performed depends on how the data are to be analysed, and the information required. Experiments vary in the method of isotope administration and in type of isotope used.

3.8.2.1 Method of isotope administration:

There are three basic methods of isotope administration - single injection, continuous infusion and primed continuous infusion, and although the accuracy is similar, the amount of information and work required vary. The attributes of each technique have been reviewed by Young (1977).

Basically, the single injection method requires frequent sampling for a period of up to 48 hours for glucose. The results

can be analysed by monoexponential analysis (fitting a straight line over part of the decay curve), multiexponential analysis (fitting a series of exponentials over the whole decay curve) or by compartmental analysis. Data obtained are turnover, pool sizes and the fraction and rate of glucose movement between pools, within the animal.

If the tracer is infused over a period, sampling time can be reduced to less than 6 hours. Information is obtained on the turnover rate but not on the internal structure of the system.

Finally the experimental time can be further reduced if the continuous infusion is preceded by an injection of tracer, called a priming dose (Shipley and Clark, 1972). The information obtained is the same as that from a continuous infusion experiment, but this method has advantages when it is necessary to minimize experimental time.

The three methods were compared, and validated for glucose metabolism in the ruminant by White et al. (1969).

3.8.2.2 Choice of isotope:

Glucose can be isotopically labelled on carbon and hydrogen atoms, and by injecting glucose labelled in different positions, different facets of metabolism can be studied by comparing the rates at which the labels are removed.

The pathway of catabolism is that glucose is released into the bloodstream, moves into interstitial fluid, diffuses into cells and is then either converted by glycolysis to lactate, which may then enter the tricarboxylic acid cycle and be completely oxidized to CO_2 and H_2O (see Fig. 1).

Thus ^{14}C appears on C_3 compounds in the tricarboxylic acid pathway and on CO_2 , while the tritium label is mainly lost as $^3\text{H}_2\text{O}$. However problems arise when some labelled compounds are resynthesized into glucose, and so glucose turnover is underestimated.

Direct reincorporation of label from $^{14}\text{CO}_2$ or $^3\text{H}_2\text{O}$ is insignificant in sheep and represents 2% and 0.5% respectively, of irreversible loss of glucose (Judson and Leng, 1972). However, only 30% of glucose is oxidized (Bergman, 1963) and so 70% is converted to other products, some of which are capable of being resynthesized into glucose. Lactate is one of these. The extent of recycling through lactate (called the Cori cycle) can be estimated by using $[\text{U-}^{14}\text{C}]$ and $[\text{6-}^3\text{H}]\text{glucose}$ (Dunn et al., 1967). The $\text{6-}^3\text{H}$ label is retained through glycolysis on pyruvate. During resynthesis of glucose from lactate, pyruvate is converted to oxaloacetate and the methyl group is lost (Fig. 1), and during equilibrium of oxaloacetate with malate and fumarate the remaining tracer is lost by proton exchanges. These reactions are called the dicarboxylic acid shuttle and result in P-enolpyruvate, and hence glucose, being free of ^3H . The rate of loss is a reflection of enzyme activities in the dicarboxylic acid shuttle.

Thus, glucose labelled $\text{U-}^{14}\text{C}$ and $\text{6-}^3\text{H}$ corrects for recycling of ^{14}C through lactate. It does not correct for recycling of ^{14}C from labelled glycogen (Katz and Dunn, 1967).

To correct for recycling via the Cori cycle, and from glycogen the use of $[\text{2-}^3\text{H}]\text{glucose}$ has been suggested (Katz and Dunn, 1967). Judson and Leng (1972) concluded that it was the preferred tracer in ruminants for calculating recycling, as it appeared in water earlier. However, this tracer is lost when glucose-6-phosphate

is inter-converted with fructose-6-phosphate (Katz and Dunn, 1967), and it was hypothesized, after experiments on dogs, that $2\text{-}^3\text{H}$ measures futile cycling, or the rate of dephosphorylation of glucose-6-phosphate, rather than the net production of glucose (Radziuk et al., 1978). The glucose is detritiated by movement of label into body water and the rate of glucose utilization is overestimated.

3.8.3 PROPOSED MODELS FOR RUMINANT GLUCOSE METABOLISM:

Compartmental models have been published for pregnant sheep (Hodgson and Mellor, 1977), lactating cows (Infield et al., 1974; Horsfield et al., 1974) and normal and hypoglycaemic cows (Kronfeld et al., 1971; Kronfeld, 1977).

A three-compartment model was postulated for non-pregnant sheep (White et al., 1969) based on the number of exponentials in the decay curve. However four compartments were distinguished from the data of twin-pregnant sheep (Hodgson and Mellor, 1974). Two of these were suggested to be plasma and extracellular glucose, and one metabolites of glucose, some of which were resynthesized into glucose. The model published to date does not include experimental detail concerning stage of pregnancy and frequency of sampling, and the precision of model parameters is not given.

The cow model of Horsfield et al. (1974) also incorporated four compartments of which two were thought to be extracellular space and the others, products and precursors which turned over at different rates. Recycling and loss were both considered to occur from compartment 3.

The model of Kronfeld et al. (1971) consisted of three compartments. The first was hypothesized to be glucose in plasma and some extracellular fluid, the second interstitial and some intracellular glucose, and the third was considered to be too large to be just glucose and was considered therefore to be products and precursors of glucose.

The model was extended by using two tracers to evaluate the extent of recycling in normal, lactating and hypoglycaemic ketotic cows (Kronfeld, 1977). To obtain a good fit to the model it was necessary to allow the recycling parameter to vary for both ^{14}C and ^3H . This conflicted with theories that ^3H does not recycle.

The compartments have yet to be defined experimentally and conflicts on recycling have yet to be resolved, before the models can have value in describing the system physiologically.

CHAPTER 2

SUMMARY OF AIMS

The aim of this study was to examine glucose metabolism in twin-pregnant ewes, before and after the induction of OPT to determine what differences exist between ewes susceptible to the disease and those not susceptible.

Four facets of metabolism were to be studied - two before and two after the induction of the disease by starvation. The ewes were to be classed as susceptible if they became recumbent with toxæmia, or non-susceptible if they failed to show symptoms after 10 days.

Firstly, glucose tolerance was to be assessed in fed twin-pregnant ewes to determine whether there is a relationship between the tolerance or insulin resistance of a ewe and her susceptibility to OPT.

Secondly, glucose kinetics were to be studied, by injecting [U-¹⁴C]- and [6-³H] glucose into the fed pregnant ewes, and analysing the results by compartmental analysis with the SAAM program (Simulation, Analysis and Modelling). The aim was initially to develop a compartmental model to explain glucose kinetics in twin-pregnant ewes, and then to fit the data for susceptible and non-susceptible ewes separately, to determine whether any differences could be detected in glucose kinetics.

Thirdly, the glucose turnover of ketotic and toxæmic ewes was to be measured after a period of starvation, by primed continuous infusion of radioactive glucose, to determine whether the in vivo glucose production rate is altered by the disease.

Fourthly, the gluconeogenic potential of the liver was to be assessed in vitro by incubating hepatocytes with gluconeogenic substrates to assess whether glucose production of ketotic sheep differs to OPT sheep.

Comparative studies were to be undertaken on starved non-pregnant sheep, to measure glucose turnover and hepatocyte glucose production.

Blood samples were to be taken from all ewes during starvation to monitor metabolite changes, to follow the development of OPT and to assess the degree of toxæmia. Liver samples were to be taken on slaughter for fat and glycogen analyses to compare the levels in starved non-pregnant, ketotic and toxæmic sheep.

Pregnancy is a prerequisite for the development of OPT, and this implicates a foetal-maternal factor in the disease. To study the foetal effect on maternal glucose metabolism, foetuses of hypoglycaemic ewes were artificially killed in utero, and the plasma glucose, glucose turnover and hepatocyte glucose production of these ewes were compared with ewes with foetuses intact.

In summary, this study aimed to examine the question posed by Kronfeld (1970), "How gluconeogenesis becomes constrained is perhaps the most important unanswered question concerning the etiology of pregnancy toxæmia."

CHAPTER 3

MATERIALS AND METHODS

1. MATERIALS

General reagents were obtained from BDH Chemicals (Poole, UK), May and Baker Ltd (Dagenham, UK) and J. T. Baker Chem. Co. (Phillipsburg, NJ, USA). Peroxidase glucose oxidase, diaphorase, bovine serum albumin, N^6, O^2 -dibutyryl adenosine 3':5'-cyclic monophosphoric acid, sodium salt, and glucagon were from Sigma Chem. Co. (St Louis, MO, USA). Amino acids were from Cal Biochem (California, USA), and human albumin was obtained from Ohra Behring Instit (Behringwerke, W. Germany). Collagenase Type CLS, activity 210 units/mg was from Worthington Biochemical Corp. (Freehold, NJ, USA), and gelatin was from Davis Gelatine (NZ) Ltd (Christchurch, NZ). Ovine insulin, crystalline, PJ4499 was from Eli Lilly (USA). Charcoal was Norit OL (activated) from Hopkin Williams (Essex, UK).

Catheters were Intracath with 16 or 14 gauge needles from Deseret Pharmaceuticals Co. (Utah, USA) or were made from polyvinyl (Dural Plastics of Eng. Pty Ltd., Dural, NSW, Australia); 0.9% saline was from Travenol (NSW, Australia); neomycin sulphate 200 mg/ml, "Neobiotic", from Upjohn Pty Ltd (NZ); heparin from Evans Medical Ltd (Liverpool, UK); procaine penicillin, 250,000 i. u. /ml and dihydrostreptomycin, 250,000 i. u. /ml (as the sulphate), "Streptopen", from Glaxo Lab. (NZ) Ltd (Palmerston North, NZ); 2-(2,6-xyllidino)-5,6 dihydro-4H-1,3-thiazine, "Rompun", 2% was purchased from Bayer (Germany); 1-butyl-2',6'-pipecoloxyllide hydrochloride, 0.5%, "Marcain" with adrenalin, and lignocaine HCl, 2%, "Xylocaine" were from Astra Chem. Pty (NSW).

Evacuated tubes for collecting blood, "Venoject" tubes (5 ml and 10 ml) were from Terumo Corp. (Japan).

D- [U- ^{14}C]-and [6- ^3H] glucose were obtained from The Radiochemical Centre (Amersham, UK) and the scintillation counting cocktail, "Aquasol", was from New England Nuclear (USA).

2. ANIMALS, DIET AND TRIAL DESIGN

2.1 PREGNANT EWES:

Experiments were performed over two years on mature Perendale Romney Crossbred ewes which were mated on known dates. At 80 days gestation they were x-rayed to determine the number of fetuses, and ewes bearing twins were moved indoors into individual pens. They were fed 40 g DM/day/kg^{0.75} of lucerne-barley pellets containing 60% lucerne, 30% barley, 5% linseed, 5% molasses and no less than 17% crude protein.

The design of the trials is outlined below for each year, and against the days of gestation are the experiments performed at that time.

1977 Glucose Kinetics, Glucose Tolerance Tests and Hepatocytes:

(23 Twin-pregnant ewes)

Days Gestation:

115 Measurement of glucose kinetics using a single injection of radioactive glucose.

117,120 Glucose tolerance tests were performed in duplicate on each ewe.

130 The starvation period began. Ewes had free access to water throughout. Blood samples were taken throughout starvation to monitor plasma metabolites. The ewes were

slaughtered for hepatocyte studies when they became recumbent with OPT; otherwise at the end of 10 days, and liver samples were collected at autopsy for fat analysis.

1978 Glucose Turnover and Hepatocytes:

(18 Twin-pregnant ewes)

Days Gestation:

130 The starvation period began and the ewes had free access to water throughout. Blood samples were taken throughout to monitor metabolite concentrations. When the ewe became recumbent with OPT, or if she survived, at the end of 10 days glucose turnover was measured using a primed continuous infusion of radioactive glucose for 3 hours. The ewe was then slaughtered for hepatocyte studies. Liver samples were collected for fat and glycogen analyses.

1978 Killing Foetuses in utero:

(8 Twin-pregnant ewes)

Days Gestation:

110 Surgery was performed to place a noose around the umbilical cord, and to implant electrodes.

120 Starvation began, and on day 4 glucose turnover was measured by giving a primed continuous infusion to all sheep. Control ewes were then slaughtered for hepatocyte studies, and the umbilical nooses were tightened on the experimental ewes. Blood samples were taken for 24 hours, then a second measurement of glucose turnover was made. After the three hour tracer infusion the ewes were slaughtered for hepatocyte studies.

2.2 NON-PREGNANT EWES:

Non-pregnant ewes (9) were housed indoors and fed the same ration as the pregnant ewes. They were then starved 1, 5 or 10 days, when glucose turnover was measured by a primed continuous infusion of radioactive glucose, and the ewes were slaughtered for hepatocyte studies. Blood samples were taken throughout starvation and analysed for glucose and acetoacetate, and liver samples were taken at autopsy for fat and glycogen analyses.

3. GLUCOSE TOLERANCE TESTS:

3.1 METHOD:

Intravenous glucose tolerance tests (IV/GTT) were performed in duplicate on 16 fed twin-pregnant ewes at about 120 days gestation, and on 4 non-pregnant ewes according to the method of Reid (1958).

A polyvinyl catheter was surgically implanted in one jugular one week prior to the experiment, and was used for both infusion of glucose solution and blood sampling.

A 50% solution of glucose in saline, previously sterilized by autoclaving was infused at the rate of 0.4 g glucose/kg. The dose was injected over one minute, flushed in with saline and the catheter rinsed by withdrawal and injection of blood. Two preinjection blood samples were taken and then at 3, 4, 5, 7, 10, 15, 20, 25, 30, 38, 45, 52, 60, 90 and 120 minutes post-injection. The 5 ml samples were stored on ice, and centrifuged at 3000 rpm for 15 min at 4°C. The plasma was analysed immediately for glucose and a portion was stored at -20°C for insulin analysis.

3.2 ANALYSES:

3.2.1 GLUCOSE:

Glucose was analysed by autoanalyser (see Blood Analyses section).

3.2.2 INSULIN

Assays of plasma from non-pregnant ewes were performed by Dr E. Payne of Ruakura Animal Research Station, Hamilton, using an Insulin RIA Kit (The Radiochemical Centre, Amersham, Buckinghamshire, England).

Plasma insulin of pregnant ewes was measured by the charcoal-separation radioimmunoassay method of Albano and Ekins (1970).

The basis of the radioimmunoassay is that insulin labelled with I^{125} binds to anti-insulin antibody found in antisera. The extent to which it binds depends on the concentration of cold, or unlabelled insulin, and therefore labelled and unlabelled insulin bind in proportion to their concentrations.

Charcoal was used to precipitate the unbound insulin, which was then counted in a gamma counter. The number of counts on the antibody was calculated by subtracting counts on the pellet from total counts added. From the standard curve of % counts bound, versus concentration, concentrations of insulin in the samples were calculated.

Tracer 'damage' was calculated by incubating labelled insulin with plasma, and determining the number of counts in the supernatant. This represented tracer which was no longer attached to insulin molecules.

3. 2. 2. 1 Solutions:

Buffer: 0.05 M Na/K Phosphate buffer pH 7.4

0.3 % HSA: Buffer + 0.3 % (w/v) Human Serum Albumin

0.1 % HSA: Buffer + 0.1 % (w/v) Human Serum Albumin

Antisera: GP78 was supplied by Princess Margaret Hospital, Christchurch, and represented guinea pig anti-porcine insulin antiserum. (Bell et al. (1970) demonstrated that these antibodies reacted with ovine insulin in a similar manner to anti-ovine antibodies.) It was diluted in 0.3 % HSA such that it bound 40-50 % added tracer in the absence of insulin.

Tracer: Porcine-insulin- I^{125} was prepared by the method of Hunter and Greenwood (1962) and was supplied by Princess Margaret Hospital for each assay and was diluted in 0.1 % HSA to 10,000 cpm/100 μ l.

Standards: Standards were made up using ovine insulin and were diluted in 0.3 % HSA to give concentrations in the range 0-200 μ U/ml.

Charcoal Suspension:

This consisted of 2 g 'Norit OL' charcoal (activated) with 180 ml 0.1 % HSA and 20 ml blank plasma, and was mixed for one hour prior to the addition to samples. The blank plasma was included to coat the charcoal with protein. This was necessary for good separation as the buffer (0.1 % HSA) had a lower protein content than plasma.

Blank Plasma:

This was plasma containing no insulin and was prepared by mixing 6 g silicic acid with 100 ml plasma. After stirring for one hour, the precipitate was allowed to

settle overnight, and the supernatant was assayed to ensure the insulin had been removed.

High Control and Low Control Standards:

These plasma samples, of high and low insulin concentration were included in each assay to determine the interassay variation.

3.2.2.2 Procedure

For each assay the following tubes were set up:

<u>Tube</u>	<u>Additions</u>
1. Total Counts	Tracer
2. Standard controls	0.1% HSA + tracer
3. Standards	0.1% HSA + standard + antisera + tracer
4. High control	0.1% HSA + high control + antisera + tracer
5. Low control	0.1% HSA + low control + antisera + tracer
6. Samples	0.1% HSA + sample + antisera + tracer

Tubes were set up in triplicate and to 100 μ l of standard/sample and 50 μ l of antisera, buffer (0.1% HSA) was added to a final volume of 700 μ l. The tubes were mixed on a Vortex and stored at 4°C for 2 days. Then 100 μ l of tracer was added to all tubes, which were vortexed and stored at 4°C for another 2 days. After this time 500 μ l of charcoal suspension was added to each tube. The tubes were mixed on a vortex and after standing at room temperature for 15 min were centrifuged at 2600 rpm for 30 min at 4°C.

The supernatant was discarded and the charcoal pellet counted for 5 min on a Packard Auto-Gamma 5110 Scintillation spectrometer. The % of counts bound were calculated, the damage value subtracted for the standards, and a standard curve was graphed.

After subtraction of the damage value the sample concentrations were read off the graph.

3.2.2.3 Interassay Variation:

The coefficients of variation between the six assays were 15% at 20 μ U/ml and 9% at 44 μ U/ml. The within assay coefficient of variation between triplicate samples was 4.6%, 21% and 21% at 4, 50 and 100 μ U/ml.

3.3 CALCULATIONS:

3.3.1 GLUCOSE TOLERANCE:

The change in plasma glucose from basal was calculated for each observation, and the results plotted against time on semilog paper.

A straight line was fitted over the 10-60 minute period using a linear regression program on a Hewlett Packard 65 calculator. The parameters of the line were used to calculate the half-life ($T_{\frac{1}{2}}$), i. e. time for half the glucose to disappear from the plasma, also called the glucose tolerance.

$$\ln Y = a_0 + a_1 X$$

Y - change in plasma glucose (mg/dl)

X - time (minutes)

$$\text{At } X = 0, \quad Y = Y_0$$

$$\text{At } T_{\frac{1}{2}} \quad \ln (Y_0/2) = a_0 + a_1 X$$

$$X = \frac{\ln (Y_0/2) - a_0}{a_1}$$

3.3.2 EXTRAVASCULAR INSULIN:

Glucose utilization is proportional to insulin concentration in the extravascular fluid, rather than plasma insulin (Sherwin et al., 1974). Using a glucose clamp system they found insulin kinetics could be predicted by a 3-compartment model, and that plasma insulin levels were not directly related to the rate of glucose utilization, but that this rate was predicted by the insulin level in a large, slowly turning over, third compartment, known as the extravascular compartment.

Arcus and McKinnon (pers. comm.) at the Christchurch Clinical School, have developed an equation using the fractional flow rates between the two compartments determined by Sherwin et al. (1974), and the plasma insulin values to predict the concentration in the third, extravascular compartment. These values agreed with those determined experimentally by Sherwin et al. (1974).

The program of Arcus and McKinnon (unpub.) was converted to run on the MAF ICL 2903 computer (Appendix 1), and, given the plasma insulin values, it calculated the extravascular values.

3.3.3 INSULIN RESISTANCE:

The insulin resistance index, R , was calculated as the product of tolerance, $T_{\frac{1}{2}}$, and extravascular insulin level, I' , when plasma glucose was 100 mg/dl above basal. This index, obtained by Arcus (unpub.) and used in the Christchurch Clinical School to discriminate between different types of diabetes, is comparable to the reciprocal of K_{ins} defined by Cunningham and Heath (1978):

$$\text{Rate of glucose disappearance} = K_{ins} \cdot \text{glucose} \cdot I'$$

where:

glucose = plasma glucose concentration (mg/dl)

Rate of glucose disappearance = $1/T_{\frac{1}{2}}$ (mg. min⁻¹.kg⁻¹)

I' = insulin in the extravascular compartment (μU/ml)

At constant plasma glucose concentration,

$$1/T_{\frac{1}{2}} = K_{ins} \cdot I'$$

$$1/K_{ins} = T_{\frac{1}{2}} \cdot I'$$

or

$$R = T_{\frac{1}{2}} \cdot I' (\mu U/ml.min)$$

R is therefore an index of the lack of sensitivity, or the resistance, of the system to glucose.

The glucose level chosen (100 mg/dl above basal) was on the linear part of the decay curve of all sheep, and, as only small quantities of glucose are excreted in the urine at this concentration (Judson and Leng, 1973a) the disappearance of glucose was attributed to the effects of insulin.

The tolerance was calculated on duplicate experiments, while plasma insulin and therefore I' and R were calculated for one experiment only, per sheep.

4. GLUCOSE KINETICS AND GLUCOSE TURNOVER:

4.1 SINGLE INJECTION TECHNIQUE

4.1.1 METHOD

The twin-pregnant sheep were housed indoors from day 80 of gestation and fed hourly to maintain steady state conditions for plasma glucose.

One week prior to the experiment a polyvinyl catheter was placed in one jugular vein using ovine cannulas with cuffs, as described by Katz and Bergman (1969a). It was flushed every second day with saline containing 10 units/ml of heparin and 0.5 ml/l

of Neobiotic. One day before the experiment an infusion catheter was inserted into the other jugular.

Infusion Solution: The isotopes, D-[U- ^{14}C] glucose (3 mCi) and D-[6- ^3H] glucose (10 mCi) were diluted separately in 100 ml of sterile 0.9% saline and stored at -20°C . The injected tracer consisted of 10 ml of each solution and was Seitz filtered into a sterile syringe and weighed. The syringe was weighed after the injection and the actual doses were determined by weight difference. About 0.3 mCi [U- ^{14}C]- and 1 mCi [6- ^3H] glucose in 20 ml was injected per animal.

Injection: The radioactive solution was injected through one catheter over 10 seconds, flushed in with sterile saline and blood was immediately sampled through the other catheter. Thirteen blood samples (10 ml) were taken over the first hour, four (15 ml) over the next two hours, and seven (20 ml) over the next 45 hours. A total of 24 samples were taken over 48 hours.

The samples were transferred to tubes containing oxalate and fluoride and stored on ice until centrifuging at 4°C for 20 min at 3000 rpm. Part of the plasma was analysed for glucose while the rest was deproteinized, for the determination of glucose specific activity.

4.1.2 ANALYSES:

4.1.2.1 Glucose:

Glucose concentrations were determined in the plasma and deproteinized plasma by autoanalyser (see Blood Analyses section).

4.1.2.2 Glucose specific activity:

The specific activity of glucose was determined on the deproteinized plasma filtrates and on dilutions of the injection solutions.

The plasma was deproteinized by the method of Somogyi (1945), by adding 15 ml of distilled water, 15 ml of 4.5% Ba(OH)_2 and 15 ml of 5% ZnSO_4 to 5 ml of plasma. Denatured protein was removed by centrifuging and then suction filtering through Whatman (No. 42) filter paper.

Glucose was isolated as potassium gluconate by the procedure of Blair and Segal (1960). Glucose carrier solution (5 ml containing 100 mg glucose) was added to 30 ml of filtrate and after drying down by rotary evaporation, the residue was extracted with three 10 ml portions of hot 90% methanol (Analar). The extracts were combined, centrifuged for 10 min and the supernatant evaporated overnight under a stream of air at 37°C . The thick syrup was dissolved in 0.2 ml of water followed by 1.2 ml of methanol. After mixing on a vortex mixer the solution was centrifuged for 30 min and the supernatant was transferred to a 25 ml beaker.

To prepare gluconate from glucose, 4 ml of iodine solution (0.285 g resublimed iodine in 4 ml of absolute methanol) was added and the mixture heated for 5 min in a water bath at 40°C . While still warm, 5.6 ml of 4% KOH (in methanol) was added, with continuous stirring until a white precipitate of potassium gluconate formed.

The solutions were left at room temperature for about two hours. The gluconate was then filtered by vacuum on to sintered glass funnels and washed with methanol, then acetone and finally dried in a desiccator.

The gluconate crystals were purified by recrystallization. The precipitate was dissolved in 1.5 ml of water, and then 60 ml of hot methanol was added followed by 20 ml of diethylether. After standing in a freezer for 30 min, the crystals were collected under vacuum on to filter paper (Whatman No. 42), washed thoroughly with methanol, then acetone and dried in a desiccator.

Crystalline gluconate was weighed into glass scintillation vials and dissolved in 3 ml of water. After the addition of 12 ml of Aquasol, the samples were counted in a Beckman Liquid Scintillation counter. The efficiency of counting was calculated from the external standard ratio and a quench correction curve, and the specific activity of glucose in the plasma sample was calculated by a program called GLUCOSESA78 (Appendix 2).

4.1.3 CALCULATIONS:

The semilog plot of specific activity (fraction of dose/mg glucose) versus time was graphed for each sheep, and several curves were peeled using a program written by Dr C. F. Ramberg for a Hewlett Packard 9815 calculator. This provided estimates of the slopes (L) and intercepts (S) for curve fitting by the program SAAM25 (Simulation, Analysis and Modelling). The animals were classified into groups (see Results, Section 1) as susceptible to OPT, or non-susceptible with live, or dead lambs. Data for the groups of animals were averaged using the program WAVE (Appendix 3).

4.1.3.1 Curve fitting:

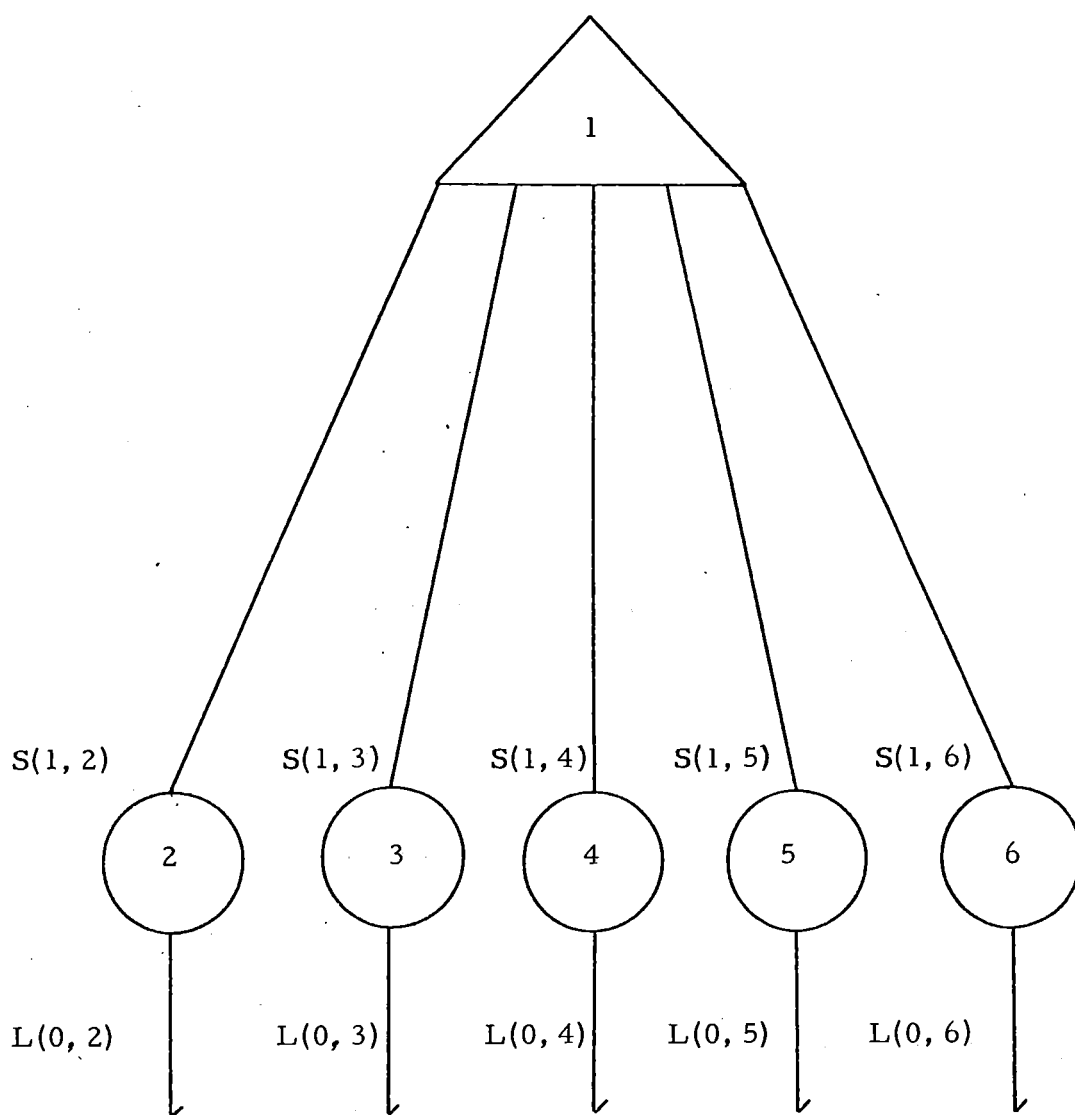
Curves were fitted for each tracer for the three groups of sheep by submitting the averaged data, estimates and initial conditions to SAAM as shown in Fig. 2. Four exponentials were obtained by curve peeling but five were fitted by SAAM.

4.1.3.2 Mapping:

The parameters of the fitted curves were used to map to a compartmental model, using the MAPPER program (Appendix 4), which converted the exponential equations for observed change in specific activity in one compartment, plasma, to parameters which represent fractional flow rates between compartments.

4.1.3.3 Model solution:

Initially the data were fitted to a series model, model A (Fig. 16),



$$F = S(1, 2)e^{-L(0, 2)t} + S(1, 3)e^{-L(0, 3)t} + S(1, 4)e^{-L(0, 4)t} \\ + S(1, 5)e^{-L(0, 5)t} + S(1, 6)e^{-L(0, 6)t}$$

Fig. 2: Schematic configuration for fitting the sum of five exponentials to tracer data.

by submitting the parameter estimates to SAAM, and describing the structure of the model by nominating flow rates. These initial estimates were improved, and the final parameter values calculated by SAAM. When an acceptable fit was obtained the steady state solution of tracee flow rates and pool sizes were calculated.

The parameters for model A were used to map to alternative models. For this model, of glucose metabolism, loss from the system was made from compartment 5, and a recycling flow was introduced back to compartment 2 (model B, Fig. 16). A physiological interpretation is discussed in the Results section.

An attempt was made to fit the data for both tracers simultaneously to the model. Thus the ^3H and ^{14}C data were fitted with only certain flow rates being allowed to adjust independently. When two experiments are performed on the same system they can be treated as a single experiment, and separated in solution by a time change, TC. Thus it was possible to test whether recycling was the only flow rate which differed between the tracers. To do this all parameters were set to be adjustable (but to be the same for both tracers), and the recycling flow rate was allowed to vary for each tracer by introducing dummy variables, P(1) and P(2). The number of flow rates allowed to vary could be increased by introducing more dummy variables.

The solution for the general model was determined, together with parameters for each group of sheep. Differences were compared in tracer movement between, and within the groups of sheep.

4.2 PRIMED CONTINUOUS INFUSIONS:

4.2.1 METHOD:

These were performed on sheep during the starvation period either when they became recumbent with OPT, or at the end of 10

days starvation. Catheters were placed into both jugular veins on the morning of the experiment.

The priming dose of 20 μCi [$\text{U}-^{14}\text{C}$] glucose and 40 μCi [$6-^3\text{H}$] glucose, in 20 ml, was injected through one catheter and immediately followed by an infusion, using a Harvard Apparatus Infusion/Withdrawal Pump (Millis, Mass, USA), of 13 μCi [$\text{U}-^{14}\text{C}$]- and 26 μCi [$6-^3\text{H}$] glucose, in 40 ml, per hour. Seven blood samples (10 ml) were taken over three hours, at 30, 60, 90, 120, 150, 165 and 180 minutes after the priming dose.

4.2.2 ANALYSES:

4.2.2.1 Plasma glucose:

Glucose was analysed in the plasma, and deproteinized plasma, by autoanalyser (see Blood Analyses Section).

4.2.2.2 Glucose specific activity:

The specific activity of glucose in the plasma and infusion solutions was determined by the method of Blair and Segal (1960) as outlined in the previous section.

4.2.3 CALCULATIONS:

The specific activity was calculated as dpm/mg by the program GLUCOESA78 (Appendix 2). The values were graphed against time, and the specific activities over the plateau period averaged. The infusion rate of isotope was determined from the activity of the infusion solution and the infusion rate. Glucose turnover was calculated:

$$\text{Turnover Rate (mg/min)} = \frac{\text{Infusion rate (dpm/min)}}{\text{Specific Activity (dpm/mg)}}$$

5. HEPATOCYTES:

5.1 ISOLATION:

Liver cells were isolated by the method of Clark et al. (1976b) which was based on the method of Berry and Friend (1969). Some modifications were introduced, however, to reduce the time required for isolation. The studies were undertaken, as outlined in Section 2.1, when the sheep became recumbent with OPT, or at the end of 10 days starvation.

The sheep was given an intravenous injection of 500 Units heparin/kg to prevent the blood from clotting. Two minutes later the sheep was stunned by a captive bolt pistol. The liver was immediately exposed, the caudate lobe removed and rinsed with Krebs-Ringer 1 (without Ca^{++}) which had been adjusted to pH 7.6 (Dawson et al., 1969). The lobe was perfused with this buffer under the same conditions as Clark et al. (1976b), using a Harvard Apparatus 1215 Variable Speed Peristaltic Pump (Millis, Mass, USA), and a constant pressure device. However only 75 mg collagenase was added to the perfusate. The perfusion lasted 30 min and upon disconnecting the lobe from the apparatus, the liver capsule was removed with forceps. The cells were able to be shaken apart in Krebs-Henseleit buffer (Dawson et al., 1969), which contained 1.5% (w/v) gelatin and was adjusted to pH 7.6. This eliminated the second incubation procedure of Clark et al. (1976b). Since calcium is involved in cell junctions it was necessary to omit it from the perfusion medium (Berry, 1976); however it was considered important to reintroduce calcium immediately the perfusion had finished to maintain cellular respiration (Howard and Pesch, 1968).

The suspension of cells was filtered through coarse nylon mesh and centrifuged at 1000 rpm for 30 sec. The cells were gently washed in Krebs-Henseleit buffer and filtered through a fine filter (100 μ m pore size). After a second wash, the cells were made up to 50 ml in Krebs-Henseleit buffer. The final suspension contained about 5 mg dry wt cells/ml. The overall preparation time was generally 60 min, and the cells were used immediately for incubation studies.

5.2 INCUBATIONS:

Incubation conditions were the same as those described by Clark *et al.* (1976b). The substrates used were Na-propionate, Li-lactate, glycerol, alanine and glutamine. These were made up in Krebs-Henseleit buffer such that the final concentration in the incubation medium was 10 mM. Glucagon was made up according to the method of Faloon and Unger (1974) and used at concentrations of 10^{-7} M or 10^{-8} M. Dibutyryl cyclic AMP (0.04 mM) was added to some of the incubation media.

The incubations were performed in triplicate and lasted 30 min. The reactions were stopped by the addition of 0.5 ml of 6% (w/v) HClO_4 , and the denatured protein was removed by centrifugation. Supernatant (1.5 ml) was neutralized with 0.1 ml of 4.5 M KOH, and after a second centrifugation it was analysed for glucose.

The viability of each cell preparation was assessed by two criteria which measured the integrity of the cell membranes.

(i) Exclusion of Trypan Blue:

Cell suspension (0.1 ml) was diluted with 0.3 ml of isotonic 0.6% trypan blue (Seglen, 1976) and the viability was assessed after five minutes, in a Burker chamber, as % of cells not stained. Only preparations with a viability greater than 80% were used.

(ii) Stimulation of Oxygen Uptake by Succinate:

This was a sensitive test for membrane integrity as only damaged membranes allowed succinate permeation at a rate sufficient to stimulate respiration, and was based on the method of Baur et al. (1975). The oxygen uptake of 1 ml of cells in Krebs-Henseleit buffer was measured polarographically with Clark apparatus (YSI Model 53 Biological Oxygen Monitor, Yellow Springs Instrument Co., Yellow Springs, Ohio, USA). The uptake was measured before and after the addition of succinate (to a final concentration of 1 mM). Only suspensions in which the oxygen uptake was stimulated by a factor less than 1.30 were used.

The dry weight of cells was determined as the difference between 2 ml cells and 2 ml buffer dried at 80°C for about 24 hours to a constant weight.

5.3 ANALYSES:

Glucose was analysed by the autoanalyser method outlined in the Blood Analyses section. For each substrate the glucose production rate was calculated from the change in glucose concentration that occurred in the medium, and was expressed as $\mu\text{mol/g}$ cells dry weight/min.

6. KILLING FOETUSES IN UTERO:

6.1 ANIMALS:

The ewes were selected as twin-pregnant at 80 days gestation and housed indoors in metabolic cages, as outlined in Section 2.1.

6.2 SURGICAL PREPARATION:

At about 110 days gestation surgery was undertaken on eight ewes to put nooses around each umbilical cord and implant two electrodes into each foetus.

The ewe was given 0.22 ml of 2% Rompun subcutaneously and five minutes later, an epidural consisting of 5 ml of 0.5% Marcain and 5 ml of 2% Xylocaine. After aseptic preparation of the skin the abdomen was opened by a 20 cm incision along the ventral midline.

The pregnant uterus was located and the foetus held while the uterus and foetal membranes were incised between cotyledons. Foetal membranes were clamped to the uterus with Allis forceps, and care was taken to minimize loss of foetal fluid while the umbilicus was located. A nylon thread was placed around the umbilicus and short (2 cm) lengths of nylon tubing were threaded on behind a 1 cm piece of a 2 ml syringe (Fig. 3). The syringe head acted as a funnel when the noose was later tightened, and the nylon tubing was sectioned to allow flexibility in the ewe, and yet still provide a rigid structure when the noose was tightened. The noose was sutured to the abdominal skin of the foetus, and electrodes were inserted subcutaneously into the hind limb and chest, and were also secured to the skin by sutures.

The uterus was closed with silk suture (3-0) and the nylon passed through longer sections of nylon tubing and pushed to the exterior under the skin using a hollow metal probe. Both foetuses were prepared in this way. The nooses and electrodes were sealed in a rubber pouch and attached to the skin of the ewe.

The ewe was given 10 ml of Streptopen intramuscularly daily for three days after surgery, and one intramuscular injection of 60 mg progesterone in olive oil to minimize the risk of abortion (Mellor and Matheson, 1975).

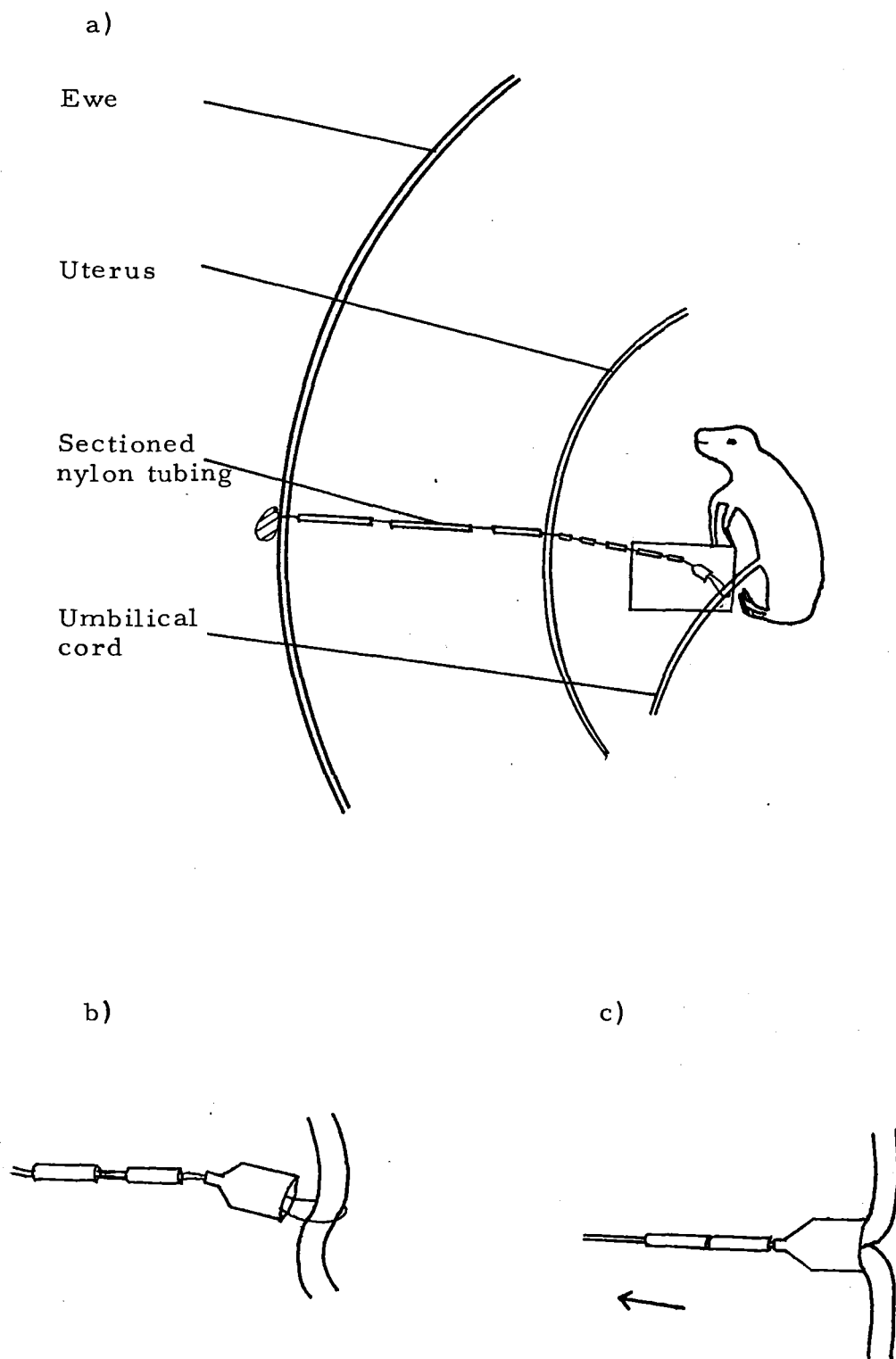


Fig. 3: Umbilical noose arrangement, a) showing placement within the ewe, b) the noose around the umbilical cord before tightening, and c) the noose after tightening showing the umbilical cord clamped off within the syringe head.

6.3 EXPERIMENTAL PROCEDURE:

Ewes were paired according to liveweight and randomly assigned to a treatment or a control group. On day four of starvation, primed continuous infusions were given to each ewe to measure glucose turnover, as outlined in Section 4.2. Control ewes were slaughtered at the end of the infusion for hepatocyte studies, while in the experimental group the foetal nooses were tightened. The foetal electrocardiogram was monitored on an oscilloscope, and heart rates were also monitored ultrasonically on Doppler equipment. Cycles of bradycardia and tachycardia occurred during the period of anoxia. Foetal death usually took 15 min from the time of tightening the nooses, and when death was established, the nooses were tied off.

Blood samples (5 ml) were taken every 10 min for the first hour, every 15 min for the second hour and every 30 min up to 24 hours. A second 3 hour infusion of tracer glucose was given as described above and then the ewe was slaughtered for hepatocyte studies.

6.4 ANALYSES:

6.4.1 BLOOD ANALYSES:

Glucose and acetoacetate were analysed as outlined in the Blood Analyses section.

6.4.2 GLUCOSE SPECIFIC ACTIVITY:

The specific activity of glucose was determined in the plasma samples and in the infusion solutions by the method of Blair and Segal (1960) as outlined previously.

6.4.3 HEPATOCYTES:

These studies were by the experimental technique outlined earlier.

6.4.4 LIVER ANALYSES:

Liver samples were analysed for fat and glycogen (see Liver Analyses section).

7. BLOOD ANALYSES:

Blood samples were collected in venoject tubes. Those for plasma analysis contained 20 mg potassium oxalate and 25 mg NaF and this mixture acted as an antiglycolytic anticoagulant. The samples were stored on ice and centrifuged at 4°C at 3000 rpm for 20 min and plasma not assayed immediately was stored at -20°C. Samples were also collected in serum tubes for analysis of creatinine, total CO₂, inorganic phosphate, protein and albumin.

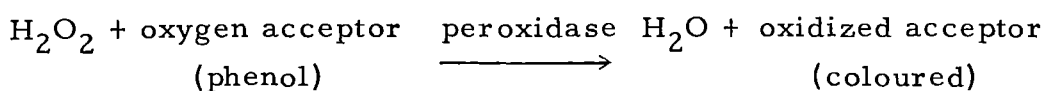
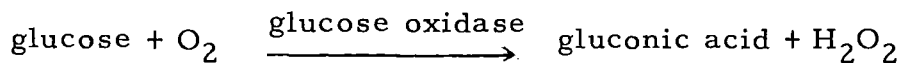
The analyses were undertaken on a Technicon Autoanalyser II, by Mr P. Dobbie.

7.1 PACKED CELL VOLUME:

This was determined on heparinized blood with a haematocrit centrifuge.

7.2 GLUCOSE:

Glucose was analysed by the method of Trinder (1969) with modifications which increased the specificity and sensitivity. These were necessary as ruminants have lower plasma glucose levels than non-ruminants. The basic reaction of the method was:



Phenol, in the presence of an oxidizing reagent gave a purple colour with 4-aminophenazone.

The main modifications in the method were that the enzymes were made up separately, azide was not included in the method as it was found to be slightly inhibitory and phenol was added to the reagent stream after the dialyser. In addition, the concentrations of aminophenazone phenol and peroxidase were increased. The solutions used were:

2% Phosphate buffer:	Na_2HPO_4 (anhydrous)	20 g
	KH_2PO_4 (anhydrous)	20 g
	in 1 l distilled water.	
Recipient solution:	0.5% 4-aminophenazone	60 ml
	2% phosphate buffer	50 ml
	18% NaCl	10 ml
	Distilled water	70 ml
	Triton-X 405	10 ml
Colour Reagent:	2% phosphate buffer	250 ml
	2% Phenol	625 ml
	Distilled water	125 ml
	Peroxidase	50 mg
Enzyme:	Glucose oxidase	500 mg
	Distilled water	100 ml
Diluent:	2% Triton X-405	

Standards:

A stock standard solution of 2 g glucose/l with 1.0 g benzoic acid was diluted to give standards in the range of 10-100 mg/dl.

The sampling rate was 50/hour with a wash time of six seconds, and the colour was read at 505 nm.

7.3 KETONES:

7.3.1 ACETOACETATE:

Acetoacetate was measured by the method of Salway (1969), with modifications (Dobbie and Wolff, unpublished), which included the use of a pure dye to increase the sensitivity of the method.

The basis of the reaction in this method was that acetoacetate reacted with 2, 5-dichlorobenzene diazonium chloride at pH 4.5 to produce formazan derivatives which were coloured.

The solutions used were:

Dye carrier:	Ethanol (absolute)	200 ml
	1 N HCl	7 ml
	Distilled water	1 l
Dye (made up daily):	2, 5-dichlorobenzene diazonium chloride	100 mg
	Dye carrier	100 ml
Buffer:	Citric acid . H_2O	19 g
	Trisodium citrate. $2H_2O$	32.7 g
	Ethylene glycol	500 ml
	Ethanol (absolute)	200 ml
	Distilled water	300 ml
	Triton-X 405	10 ml
10% (w/ v) NaOH		
Diluent:	2% (v/v) Triton-X 405	
Recipient:	HCl	4.4 ml
	Triton-X 405	20 ml
	made up to 1 l in distilled water.	

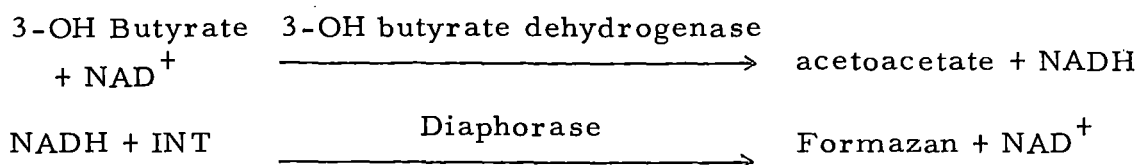
Standards:

A stock standard solution of 2 mM Li-acetoacetate was diluted to give standards of 0.1-1.4 mM.

The sample rate was 50 per hour, with a wash time of seven seconds and the optical density was read at 520 nm.

7.3.2 3-HYDROXYBUTYRATE:

This was analysed by the method of Zivin and Snarr (1973) which was adapted for the Technicon Autoanalyser II. The basic reactions of this method were:



(INT = 3-p-nitrophenyl-2-p-iodophenyl-5-phenyltetrazolium chloride)

The change in absorbance due to the reduction of INT was proportional to the concentration of 3-hydroxybutyrate in the sample.

The solutions used were:

Recipient buffer:	Tris	12.5 g
	NaCl	12.4 g
	Semicarbazide HCl	2.22 g
	EDTA (Ethylene diamine tetra-acetic acid)	3.5 g
	Triton-X 405	50 ml
	made up to 1 l in distilled water.	
Enzyme solution:	Diaphorase	5 mg
	Bovine serum albumin (Fraction V)	15 mg
	NAD	220 mg
	Buffer	50 ml
	3-OH-butyrate dehydrogenase	2 mg

0.06% INT (w/v)

2% Triton-X 405

Standards:

A stock standard solution (20 mM) was made up of 5.04 g 3-OH-butyrate (Na salt)/l, which was double strength as it contained a mixture of D- and L- isomers but only the L-form was detected enzymatically. The stock was diluted to give working standards of 1-10 mM.

Samples were run at 50 per hour, with a wash time of six seconds, and the optical density was read at 505 nm.

7.4 FREE FATTY ACIDS:

Plasma free fatty acid determinations were undertaken by Miss B. Crane of the Chemical Services Section of Ruakura, using the semiautomated technique of Crane and Lane (1977).

The free fatty acids were extracted in an isopropanol-n-heptane-1 N H₂SO₄ mixture (40:10:1) and volatile acids, especially CO₂, were removed by bubbling with nitrogen. The heptane phase was washed with 0.05% (v/v) H₂SO₄, and the extracts were stored at 40°C until analysed.

Palmitic acid standards, in the range 160-1440 µmol/l, were extracted with each group of plasma samples.

The analysis was performed on an Autoanalyser I, and the basis of the reaction was:



The pH indicator, phenol red changed colour as the pH of the solution decreased, and this colour change was measured at 550 nm.

7.5 UREA:

Urea was measured by the standard Technicon method (Method 01), based on the work of Marsh et al. (1965). Urea reacted with diacetyl-monoxime in weak acid solution to form a coloured product. The colour, which was intensified with thiosemicarbazide and with ferric ions, developed at 90°C and was read at 520 nm.

The solutions used were:

Colour reagent:	Diacetyl-monoxime 2.5% (w/v)	67 ml
	Thiosemicarbazide 0.5% (w/v)	67 ml
	Brij-35 30%	1 ml
	Distilled water to 1 l.	

Ferric chloride-phosphoric acid:

$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	15 g
Phosphoric acid 85%	300 ml
Distilled water to 450 ml.	

Acid solution:	Ferric chloride-phosphoric acid	1 ml
	H_2SO_4 20% (v/v) to 1 l.	

Standards:

Were prepared in the range of 10-80 mg/dl.

7.6 CREATININE:

Creatinine was measured by the standard clinical method No. SE2-0011FC4 for Technicon Autoanalyser II, based on the method of Chasson et al. (1961) which used the Jaffe reaction in which creatinine reacted with alkaline picrate to give a red colour, measured at 505 nm.

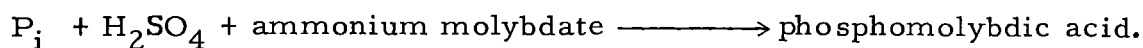
The reagents used were:

- 1.8% NaCl (w/v)
- 0.5 N NaOH
- Picric acid 1.3% (w/v)
- Brij-35 0.03% (w/v)
- Standards 0-20 mg/dl

7.7 INORGANIC PHOSPHATE:

This was analysed by the Technicon Autoanalyser II clinical method No. 4 which is based on the methods of Hurst (1967) and Kraml (1966).

The basic reaction was:



The product was reduced by stannous chloride-hydrazine, and the absorbance read at 660 nm. The solutions used were:

H₂SO₄ 0.36 N (with Levor IV)

Ammonium molybdate 1% (in HCl)

1% Hydrazine sulphate

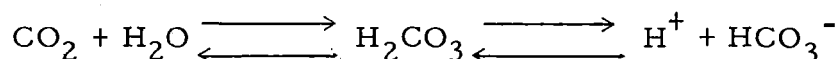
Stannous chloride: SnCl₂ · 2H₂O 400 mg
made up to 10 ml with conc. HCl.

Stannous chloride-hydrazine:

Stannous chloride 4 ml
made up to 500 ml with 1% hydrazine sulphate.

7.8 TOTAL CARBON DIOXIDE:

Total CO₂ was analysed by the Technicon AAI clinical method No. 11-08 which was based on the method of Skeggs and Hochstrasser (1964). In the plasma the following equilibrium existed:



Acid forced the reaction to the left, and so the method measured CO₂ and HCO₃⁻ in the plasma, or total CO₂. The sample was mixed with a CO₂-free, air-segmented acid solution and heated to 37.5°C, releasing CO₂ into the gaseous phase. The CO₂ then diffused across a silicone-rubber dialyser membrane into a solution containing the pH indicator Cresol red. As the pH decreased the colour change was read at 420 nm.

The solutions used were:

Buffer pH 10.0:	1 M Tris (121 g/l)	900 ml
	1 M NH ₄ · OH	100 ml
	pH adjusted with conc. HCl	
Sulphuric acid:	H ₂ SO ₄ conc.	13.9 ml
	made up to 1 l with distilled water.	
	Brij-35 30%	1 ml

CO ₂ Colour reagent:	Cresol Red 1% (w/v)	4 ml
	Buffer, pH 10.0	3 ml
	made up to 1 l with distilled water, pH 9.2	
	Brij-35 30%	1 ml

Standards:

Were prepared in the range 10-50 mM.

7.9 TOTAL PLASMA PROTEIN:

The Technicon Autoanalyser method, AA II-14, was based on a modified Biuret reaction in which copper in alkaline solution reacted with peptide linkages of amino acids in the proteins to form a purple complex. The absorbance was then measured at 550 nm. The solutions used for this method were:

Biuret Stock in NaOH 0.2 N:

Sodium potassium tartrate	45 g
CuSO ₄ · 5H ₂ O	15 g
KI	5 g
NaOH	8 g
made up to 1 l in distilled water.	

Biuret Working Solution:

Biuret stock in NaOH 0.2 N	200 ml
made up to 1 l in 0.5% KI (in 0.2 N NaOH).	
Wetting agent ARW-7	1 ml

Total Protein Blank solution:

KI 0.5% in 0.2 N NaOH	800 ml
Distilled water	200 ml
Wetting agent ARW-7	1 ml

7.10 ALBUMIN:

Albumin was analysed by the Bromocresol Green (BCG) Auto-analyser method No. TN3-0160-20 which was based on the method of Doumas et al. (1971). When albumin was added to a solution of BCG it caused a change in colour which was proportional to the

amount of albumin present, and the BCG-albumin complex was read at 630 nm. The method was very sensitive, and so the sample was first diluted in 0.03% Brij-35, a detergent, which prevented turbidity.

The reagents used in the method were:

BCG Dye, pH 4.2 in Succinate buffer
(Technicon Product No. T01 -0573)

Diluent: 0.03% Brij-35

8. LIVER ANALYSES:

8.1 FAT

Fat analyses were performed by Miss B. Crane of the Chemical Services Section, Ruakura. The liver was homogenized in a Sorvall blender and about 10 g of the homogenate was weighed and freeze dried. The dry weight was determined and the sample refluxed in soxhlet thimbles with petrol ether, b.p. 60-80°C for six hours in a multiple extractor unit.

The soxhlet thimble was dried and weighed, and the weight loss represented the fat content.

8.2 GLYCOGEN:

Glycogen was extracted from the liver with water and phenol by the method of Laskov and Margoliash (1963), and analysed by the iodine/iodide method of Krisman (1962) which was based on the quantitative reaction of the I_2/I^- reagent with polysaccharide. Calcium chloride enhanced the colour and absorption of the chromophore, and so increased the sensitivity.

A stock iodine/iodide solution was made up by dissolving 2.64 g iodine and 26.4 g KI in 100 ml of water. This solution was stored

at 4°C in the dark. The working solution was made by dissolving 2 ml of stock in 1 l of saturated CaCl_2 , and this was stored in a dark bottle in a warm room. Other solutions used were 0.15 M Tris Buffer, adjusted to pH 8.2 with HCl, and 90% (w/v) Phenol.

Duplicate liver samples of about 2 g were finely chopped and weighed into 5 ml of Tris Buffer. The samples were homogenized with a teflon pestle for about a minute, and then 5 ml of 90% phenol was added. After vortex mixing, the samples were centrifuged at 3000 rpm for 30 minutes.

The aqueous phase of the supernatant was pipetted off, and the remaining phenol phase was washed with 5 ml of water, and recentrifuged for 30 minutes. The aqueous phases were combined.

The glycogen was precipitated with 40 ml of ethanol, and some CsCl granules. The glycogen was sedimented by centrifuging at 3000 rpm for 30 minutes and was oven dried at 60°C for 5 minutes. It was made up to 20 ml with water.

To measure the glycogen concentration, 0.5 ml of the sample was mixed with 3.25 ml of iodine/iodide working solution, and read on a Gilford 300M or Gilford 2400S spectrophotometer at 400 nm. The samples were read against a water-plus-reagent blank.

Standards were prepared by the phenol/water extraction of 5 g fresh liver which had been frozen in liquid N_2 . The glycogen was prepared in the same way as for the samples, but was purified by precipitating with ethanol five times. After drying in a desiccator a series of standards were prepared in the range of 0.1-2.0 mg/ml. These were used to prepare a standard curve from which the unknown sample concentrations were read.

9. STATISTICAL ANALYSES:

The mean, standard deviation and variance were calculated for all data, and for any group comparison the variances were tested for equality by the F-test. If the test was not significant the means were assumed to be from the same normally distributed population, and data were compared by the "Student's" t-test for independent samples. If the F-test was significant, the means were compared by the non-parametric Wilcoxon's two-sample rank test. Treatment effects within a group were tested by the non-parametric Chi-squared test.

The calculations on metabolites were by the SPSS (Statistical Package for the Social Sciences) and BMD-Biomedical computer programs on the Canterbury University Burroughs B6700 computer.

CHAPTER 4

RESULTS

1. CLASSIFICATION OF SHEEP

Sheep were classified as susceptible (S) if they became recumbent with toxæmia, or non-susceptible (NS) if they survived a 10 day starvation period without showing symptoms of the disease. They were further classified according to the lambs being dead (D) or live (L) at the time the ewe was slaughtered.

Ewes which showed signs of OPT but which were not recumbent with the disease were classified susceptible if signs of renal failure were evident. Such signs were plasma urea >80 mg/dl and plasma creatinine >3 mg/dl.

When tested by the BMD program and multilinear regression, the year was found to have had a significant effect on the distribution of sheep between groups, and so results of the two years were analysed, and are presented separately.

2. EFFECT OF STARVATION ON PLASMA AND LIVER METABOLITES.

2.1 PREGNANT EWES - RELATIONSHIP TO OPT:

Changes in blood metabolites were monitored throughout starvation, as indicators of the metabolic status of the ewes. Blood samples were taken on alternate days in the first trial, and daily in the second.

Results for the first seven days starvation for all groups in 1977 are shown in Table 3, and for 1978 in Table 4.

The final blood and liver metabolite levels at slaughter are shown in Table 5.

TABLE 3: 1977: Effect of Starvation on Blood Metabolite Concentrations of Twin-Pregnant Ewes.

Metabolite Group	Days Starvation			
	0	3	5	7
PCV (%)				
S-L	33 ± 5	35 ± 4	34 ± 6	34 ± 5 ^a
NS-L	34 ± 2	35 ± 3	36 ± 3	37 ± 2 ^{ab}
NS-D	36 ± 3	36 ± 4	39 ± 2	40 ± 3 ^b
Glucose (mg/dl)				
S-L	48 ± 11	31 ± 15 ^{ab}	29 ± 18	36 ± 21
NS-L	53 ± 6	22 ± 2 ^a	23 ± 4	26 ± 7
NS-D	56 ± 11	28 ± 4 ^b	28 ± 9	37 ± 22
3-OH Butyrate (mM)				
S-L	0.9 ± 0.5	3.7 ± 1.9	5.8 ± 2.0	6.4 ± 2.2
NS-L	0.5 ± 0.1	3.3 ± 1.7	4.2 ± 1.7	4.5 ± 1.9
NS-D	1.0 ± 0.6	3.5 ± 1.6	5.7 ± 2.0	7.1 ± 3.0
Acetoacetate (mM)				
S-L	0.2 ± 0.2	0.9 ± 0.4	1.4 ± 0.6	1.5 ± 0.6
NS-L	0.1 ± 0.0	0.9 ± 0.4	1.3 ± 0.5	1.3 ± 0.6
NS-D	0.1 ± 0.1	0.8 ± 0.2	1.3 ± 0.5	1.3 ± 0.6
Urea (mg/dl)				
S-L	28 ± 7	51 ± 8	60 ± 23	87 ± 28
NS-L	25 ± 9	54 ± 19	50 ± 19	59 ± 28
NS-D	31 ± 7	49 ± 13	59 ± 16	128 ± 90
Creatinine (mg/dl)				
S-L	0.9 ± 0.2	1.1 ± 0.2	1.3 ± 0.5	1.6 ± 0.5 ^{ab}
NS-L	0.9 ± 0.1	1.2 ± 0.2	1.2 ± 0.2	1.3 ± 0.2 ^a
NS-D	0.8 ± 0.1	1.2 ± 0.4	1.3 ± 0.4	2.3 ± 1.1 ^b
TCO ₂ (mM)				
S-L	26 ± 2	21 ± 4	19 ± 3 ^a	16 ± 7 ^{ab}
NS-L	26 ± 3	21 ± 3	22 ± 2 ^b	22 ± 4 ^a
NS-D	26 ± 5	20 ± 3	18 ± 3 ^{ab}	15 ± 5 ^b
Albumin (g/dl)				
S-L	3.3 ± 0.4	3.3 ± 0.3	3.4 ± 0.4	3.6 ± 0.4
NS-L	3.4 ± 0.4	3.4 ± 0.4	3.6 ± 0.6	3.4 ± 0.7
NS-D	3.5 ± 0.2	3.5 ± 0.0	3.7 ± 0.1	3.7 ± 0.5
Total Plasma Protein (g/dl)				
S-L	6.5 ± 0.5	6.5 ± 0.6	7.0 ± 0.7	7.4 ± 0.7
NS-L	6.7 ± 0.5	6.7 ± 0.6	7.2 ± 0.6	7.4 ± 0.6
NS-D	6.8 ± 0.3	6.8 ± 0.3	7.4 ± 0.5	7.9 ± 0.5
Inorganic Phosphate (mg/dl)				
S-L	5.3 ± 1.2	8.1 ± 1.9	8.8 ± 2.4	11.2 ± 2.5 ^a
NS-L	4.4 ± 1.1	7.1 ± 2.1	7.2 ± 2.0	7.8 ± 2.0 ^b
NS-D	4.5 ± 0.6	8.0 ± 1.5	8.7 ± 2.8	12.2 ± 4.8 ^a
Free Fatty Acids (mM)				
S-L	1.1 ± 0.6	1.4 ± 0.3	1.4 ± 0.5 ^a	1.3 ± 0.4 ^a
NS-L	0.5 ± 0.2	1.5 ± 0.6	1.8 ± 0.3 ^b	1.8 ± 0.3 ^b
NS-D	0.4 ± 0.2	1.8 ± 0.3	1.4 ± 0.2 ^a	1.2 ± 0.3 ^a
		Mean ± S.D.		
Number of Sheep				
S-L	11	11	11	(9)
NS-L	7	7	7	7
NS-D	4	4	4	4

Means with different superscripts differ at 5% level based on the t-test.

Ewes were susceptible (S) or non-susceptible (NS) to pregnancy toxemia with live (L) or dead (D) lambs in utero at slaughter.

TABLE 4: 1978: Effect of Starvation on Blood Metabolite Concentrations of Twin-Pregnant Ewes.

Metabolite Group	Days Starvation						
	0	1	2	3	4	5	7
PCV (%)							
S-L	36 ± 1 ^a	37 ± 6	35 ± 4	34 ± 5	32 ± 3	34 ± 4 ^{ab}	34 ± 4
S-D	33 ± 1 ^b	35 ± 1	35 ± 1	34 ± 2	33 ± 2	36 ± 1 ^a	40 ± 5
NS-L	33 ± 3 ^{ab}	36 ± 1	36 ± 3	35 ± 4	35 ± 3	33 ± 1 ^b	36 ± 2
NS-D	34 ± 8 ^{ab}	40 ± 5	38 ± 6	35 ± 2	34 ± 3	34 ± 2 ^{ab}	36 ± 3
Glucose (mg/dl)							
S-L	44 ± 9	37 ± 3	34 ± 7	26 ± 6	27 ± 9	21 ± 5 ^{ab}	31 ± 10
S-D	45 ± 8	33 ± 8	35 ± 10	24 ± 3	26 ± 4	27 ± 5 ^{ab}	83 ± 21
NS-L	45 ± 4	34 ± 3	33 ± 1	22 ± 5	30 ± 5	33 ± 6 ^a	41 ± 24
NS-D	51 ± 5	38 ± 7	31 ± 5	26 ± 7	24 ± 2	24 ± 3 ^b	34 ± 14
3-OH Butyrate (mM)							
S-L	0.3 ± 0.0	0.9 ± 0.2	1.8 ± 0.4	2.6 ± 0.5	3.5 ± 0.1	4.1 ± 0.3	3.5 ± 0.3
S-D	0.3 ± 0.0	0.8 ± 0.2	1.3 ± 0.3	2.3 ± 0.7	3.1 ± 0.9	3.5 ± 1.1	2.4 ± 0.7
NS-D	0.4	0.9	2.2	3.3	3.9	5.2	1.5
Acetoacetate (mM)							
S-L	0.1 ± 0.0	0.4 ± 0.1	0.8 ± 0.1 ^{ab}	1.2 ± 0.1 ^{ab}	1.7 ± 0.1 ^{ab}	1.9 ± 0.5	1.3 ± 1.0 ^{ab}
S-D	0.1 ± 0.0	0.4 ± 0.3	0.6 ± 0.3 ^a	0.9 ± 0.3 ^a	1.1 ± 0.5 ^a	1.2 ± 0.8	0.7 ± 0.5 ^a
NS-L	0.1 ± 0.0	0.3 ± 0.1	0.8 ± 0.3 ^{ab}	1.1 ± 0.6 ^{ab}	2.1 ^{ab}	1.0 ± 0.9	2.2 ± 0.9 ^b
NS-D	0.1 ± 0.0	0.3 ± 0.1	1.0 ± 0.3 ^b	1.6 ± 0.4 ^b	2.0 ± 0.4 ^b	1.5 ± 0.8	0.6 ± 0.4 ^a
Urea (mg/dl)							
S-L	37	46	49	41		50	42
S-D		40	55	69	94		
NS-L	31 ± 6	31 ± 6	37 ± 8	42 ± 14	37 ± 10	40 ± 8	33 ± 5
NS-D	33 ± 1	37 ± 3	45 ± 8	41 ± 7	38 ± 5	40 ± 8	31 ± 17
Creatinine (mg/dl)							
S-L	1.0 ± 0.1	1.1 ± 0.2	1.1 ± 0.1	1.1 ± 0.2	0.9 ± 0.1 ^{ab}	1.6 ± 0.5	2.1 ± 1.1
S-D	0.9 ± 0.1	1.0 ± 0.2	1.3 ± 0.3	1.3 ± 0.3	1.6 ± 0.6 ^{ab}	2.3 ± 1.5	4.0 ± 3.7
NS-L	0.9 ± 0.0	1.1 ± 0.1	1.1 ± 0.1	0.9 ± 0.3	0.7 ± 0.2 ^a	1.2 ± 0.2	1.3 ± 0.2
NS-D	1.0 ± 0.1	1.2 ± 0.2	1.4 ± 0.2	1.2 ± 0.3	1.2 ± 0.2 ^b	1.6 ± 0.6	2.6 ± 2.4
Mean ± S.D.							
Number of Sheep							
S-L	3	3	3	3	3	3	3
S-D	6	6	6	6	6	5	5
NS-L	3	3	3	3	3	3	3
NS-D	5	5	5	5	5	5	5

NB: Urea and 3-OH Butyrate were not done on all sheep.

Means with different superscripts differ significantly at 5% level, based on the t-test.

Ewes were susceptible (S) or non-susceptible (NS) to pregnancy toxæmia with live (L) or dead (D) lambs in utero at slaughter.

TABLE 5: Blood and Liver Metabolites of Starved Pregnant Ewes at Slaughter.

1977

Group	No.	PLASMA							
		3-OH B (mM)	Ketones (mM)	Urea (mg/dl)	FFA (mM)	TCO ₂ (mM)	Alb (g/dl)	TPP (g/dl)	PI (mg/dl)
S-L	11	7.1 ± 3.6 ^a	8.5 ± 4.1 ^a	102 ± 76 ^{ab}	1.2 ± 0.4 ^a	17 ± 5 ^a	3.7 ± 0.5	7.5 ± 0.8	11 ± 4 ^a
NS-L	8	5.9 ± 2.6 ^a	7.2 ± 3.1 ^a	86 ± 29 ^a	1.4 ± 0.5 ^a	19 ± 5 ^a	3.6 ± 0.6	7.8 ± 0.7	9 ± 2 ^a
NS-D	4	2.1 ± 1.8 ^b	2.5 ± 2.2 ^b	250 ± 123 ^b	0.6 ± 0.1 ^b	11 ± 3 ^b	3.9 ± 0.4	7.7 ± 0.7	17 ± 4 ^b

1977

Group	No.	PLASMA			LIVER
		Glucose (mg/dl)	AcAc (mM)	Creat (mg/dl)	Fat (% wet weight)
S-L	11	40 ± 22 ^a	1.4 ± 0.7 ^a	2.0 ± 1.1	16 ± 6 ^a
NS-L	8	26 ± 10 ^a	1.3 ± 0.7 ^a	1.5 ± 0.6	20 ± 7 ^{ab}
NS-D	4	82 ± 12 ^b	0.4 ± 0.4 ^b	4.7 ± 2.7	9 ± 3 ^b

1978

Group	No.	PLASMA			LIVER	
		Glucose (mg/dl)	AcAc (mM)	Creat (mg/dl)	Fat (% wet weight)	Glycogen (mg/g)
S-L	4	57 ± 28 ^{ab}	1.4 ± 0.8	4.4 ± 2.7 ^a	8 ± 5	2.0 ± 0.5
S-D	9	111 ± 102 ^{ab}	1.5 ± 1.7	4.1 ± 3.7 ^a	12 ± 11	2.1 ± 1.4
NS-L	3	38 ± 15 ^a	1.6 ± 1.4	1.6 ± 0.2 ^b	16	1.8
NS-D	6	119 ± 75 ^b	0.3 ± 0.2	8.4 ± 8.1 ^{ab}	6 ± 2	18.1 ± 10.3 ^b

Mean ± S.D.

Means with different superscripts differ significantly at 5% level, based on Wilcoxon's two-sample rank test.

Ewes were susceptible (S) or non-susceptible (NS) to pregnancy toxæmia, with live (L) or dead (D) lambs in utero at slaughter.

AcAc - Acetoacetate
Alb - Albumin
3-OH B - 3-Hydroxybutyrate
Creat - Creatinine

FFA - Free fatty acids
PI - Inorganic phosphate
TCO₂ - Total CO₂
TPP - Total plasma protein

2.1.1 PACKED CELL VOLUME

This is an indicator of dehydration and the levels tended to rise throughout starvation (Fig. 4). In 1977 a difference between groups was detected after 7 days of starvation when NS-D had significantly higher levels than S-L.

In 1978 the results showed greater variability and there were no consistent differences between the groups. The levels rose from 33-36% to 34-40% with the largest increase (7%) in the S-D group.

2.1.2 GLUCOSE:

On starvation all ewes became hypoglycaemic (less than 30 mg/dl) and plasma glucose reached a minimum on day 3 (Fig. 5). The prestarve concentration was 45-55 mg/dl and the minimum was 25 mg/dl. After day 5 glucose concentrations increased in all groups, and some ewes became hyperglycaemic (more than 80 mg/dl).

In 1977 the S-L group showed greater variability in plasma glucose from day 3 onwards, while the NS-L group reached low levels and tended to be lower for the rest of the starvation period. The NS-D group had significantly higher levels than the NS-L group and had become hyperglycaemic by the time of slaughter.

The 1978 results tended to follow the same pattern as 1977 with levels reaching a minimum value after 3-4 days of starvation. No difference was detected on the depth of hypoglycaemia between the groups. In contrast to the previous year, the NS-L group had the highest plasma glucose after day 3. At the time of slaughter, the groups with dead lambs had become hyperglycaemic. The groups with live lambs had mean values in the normal range. One ewe with one live lamb was hyperglycaemic.

Thus hypoglycaemia occurred in all ewes on starvation and preceded neurological symptoms of OPT. However it was not

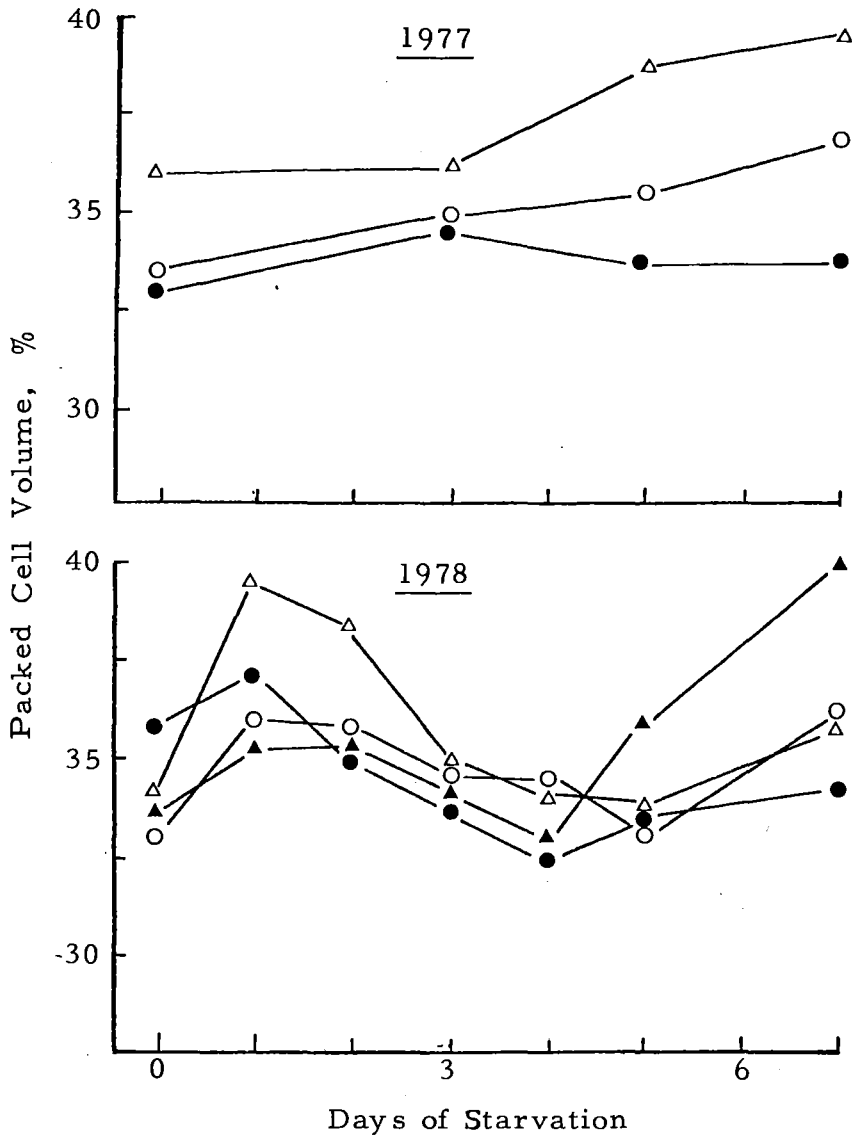


Fig. 4: Changes in packed cell volume during starvation of twin-pregnant ewes. Ewes were susceptible (S) or non-susceptible (NS) to toxæmia, and at slaughter had live (L) or dead (D) lambs.

S-L (●)	NS-L (○)
S-D (▲)	NS-D (△)

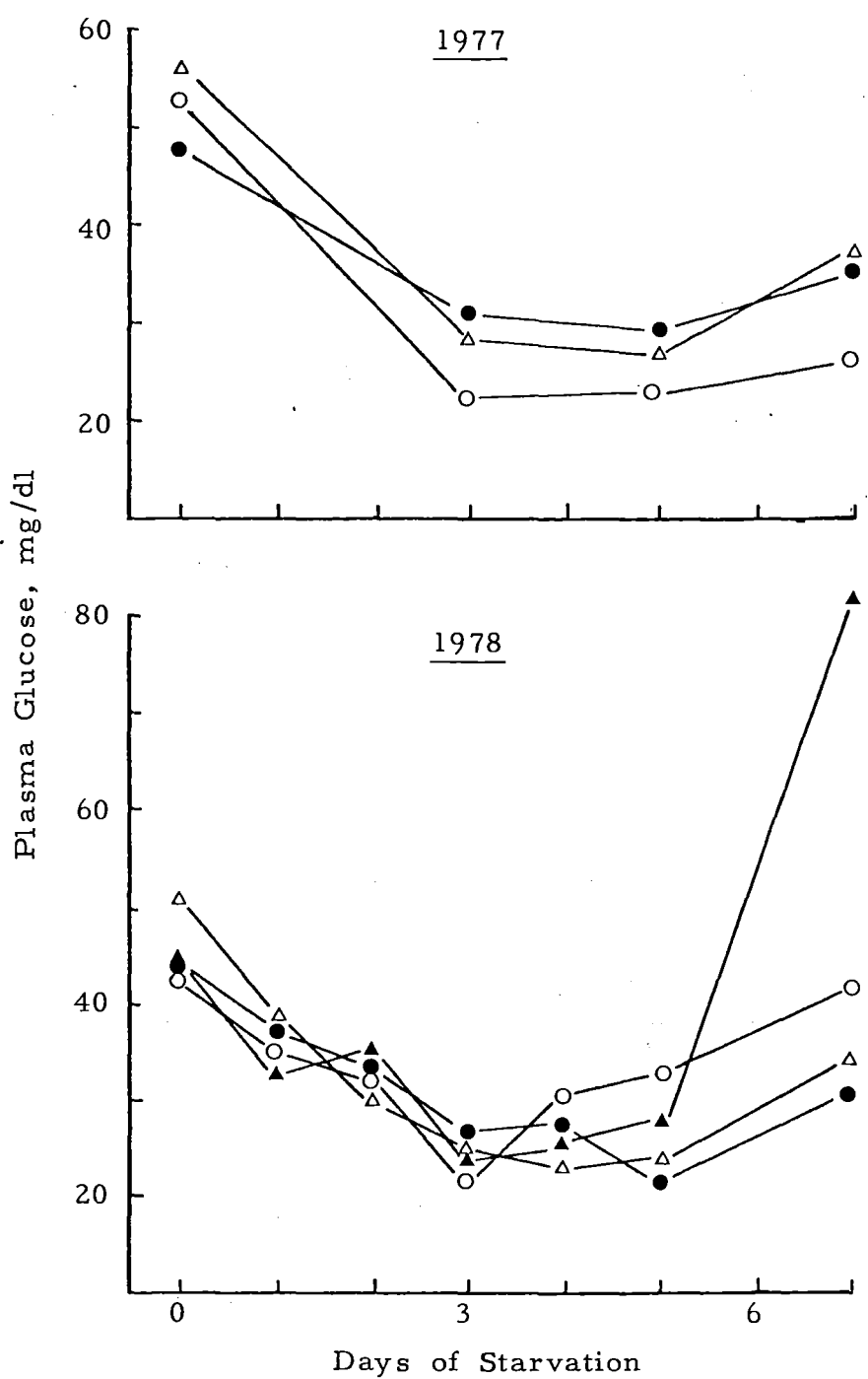


Fig. 5: Changes in plasma glucose during starvation of twin-pregnant ewes. Ewes were susceptible (S), or non-susceptible (NS) to toxæmia, and at slaughter had live (L) or dead (D) lambs.

S-L (●)	NS-L (○)
S-D (▲)	NS-D (△)

possible to distinguish between S and NS ewes based on plasma glucose concentrations. Hyperglycaemia was commonly seen in ewes with dead lambs both in the absence and presence of the signs of OPT.

2.1.3 3-OH BUTYRATE:

The level of this ketone body increases with fat catabolism. The levels rose in both years during starvation, from prestarve levels of < 1 mM to a maximum of 4-6 mM by day 5. There were no significant differences between groups although the NS-L group tended to have the lowest levels.

A different pattern was seen each year (Fig. 6) with levels reaching a plateau after day 5 in 1977, but declining in 1978. On slaughter the NS-D group had significantly lower values than the other groups.

2.1.4 ACETOACETATE:

This ketone body also rose from a prestarve value of < 1 mM to a maximum of 1-2 mM after 5 days of starvation (Fig. 7).

No differences were detected between the groups in 1977; however in 1978 the NS-D group had significantly higher levels than the S-D on days 2, 3 and 4 of starvation. After day 4 levels decreased in all groups except the NS-L. At the time of slaughter the NS-D group had significantly lower levels, while the means of the other groups were similar.

Thus acetoacetate levels increased with the degree of starvation but NS and S groups could not be distinguished on the rate that the concentrations increased or on the maximum values attained.

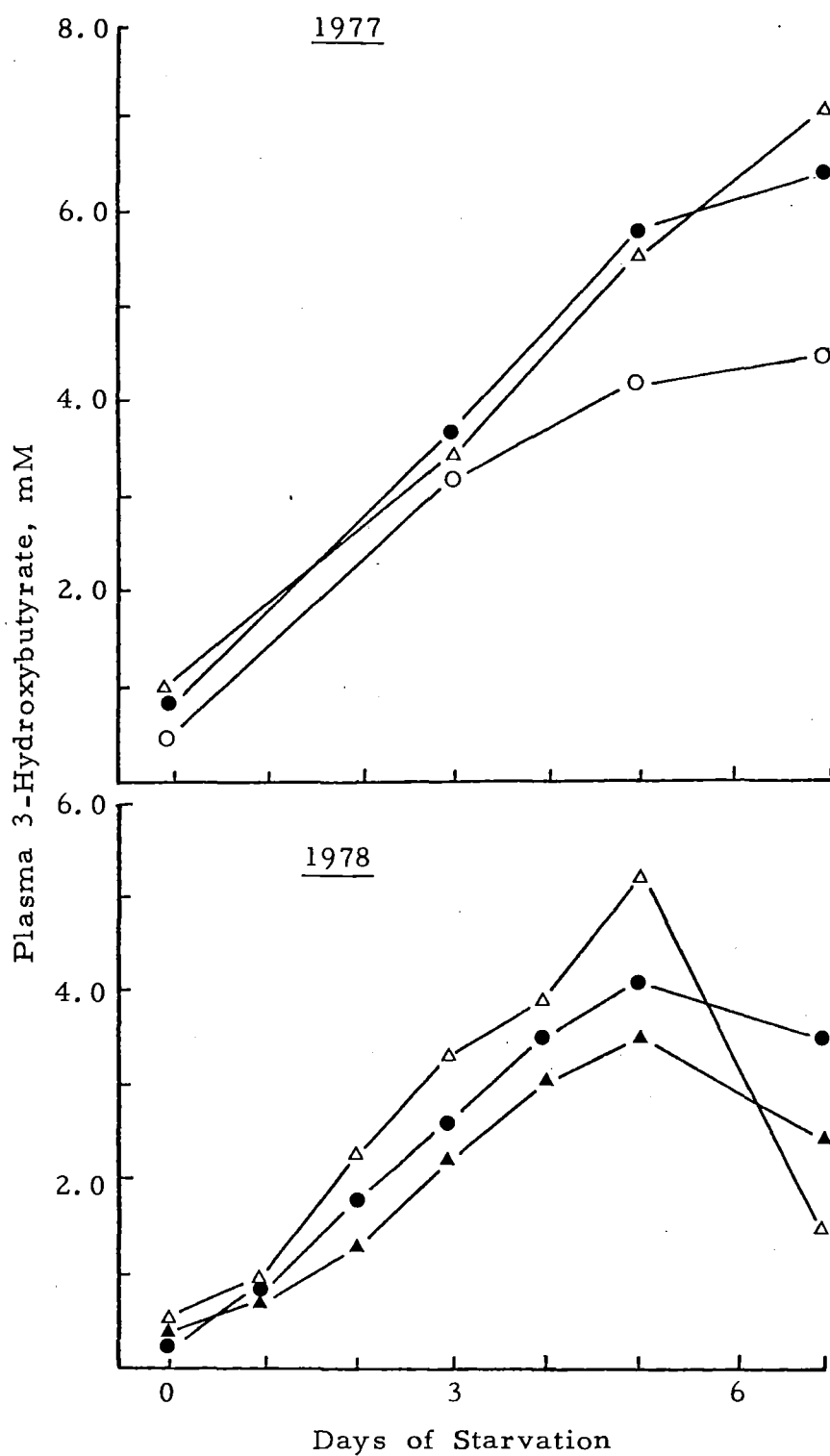


Fig. 6: Changes in plasma 3-hydroxybutyrate during starvation of twin-pregnant ewes. Ewes were susceptible (S) or non-susceptible (NS) to toxæmia, and at slaughter had live (L) or dead (D) lambs.

S-L (●)

NS-L (○)

S-D (▲)

NS-D (△)

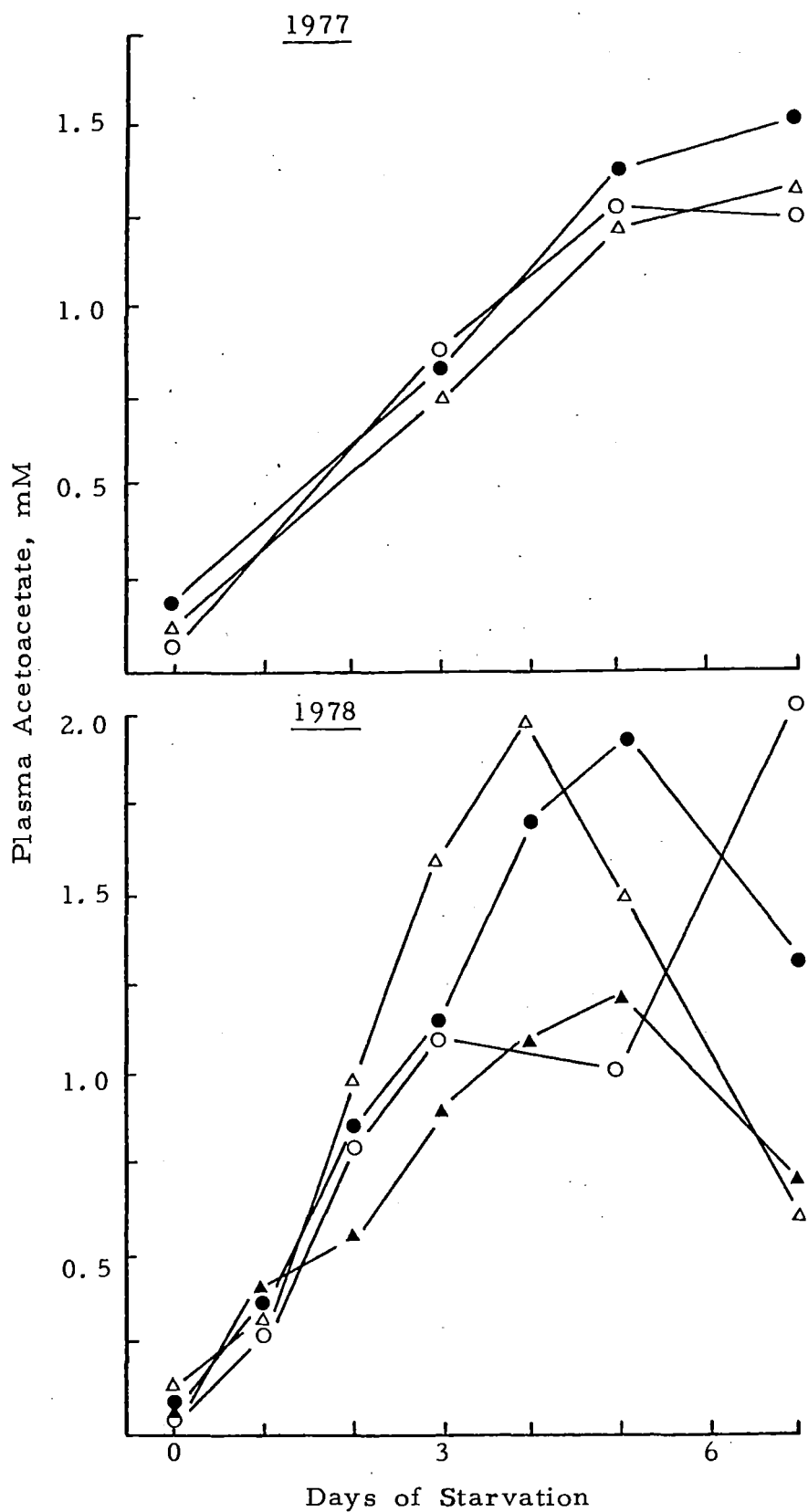


Fig. 7: Changes in plasma acetoacetate during starvation of twin-pregnant ewes. Ewes were susceptible (S) or non-susceptible (NS) to toxaemia, and at slaughter had live (L) or dead (D) lambs.

S-L (●)	NS-L (○)
S-D (▲)	NS-D (△)

2.1.5 FREE FATTY ACIDS:

Plasma free fatty acid concentrations indicate the level of fat mobilization. The values increased dramatically (3-4 fold) in all groups (Fig. 8), in the first 3 days of starvation.

In the NS-D group levels peaked at day 3 and then declined, whereas in the NS-L group the levels continued to rise up to days 5 and 7. On slaughter, however, there was no difference between S and NS groups with live lambs, while NS-D had significantly lower levels.

2.1.6 UREA:

Urea level is an indicator of kidney function, and impaired clearance is generally due to reduced glomerular filtration rate.

The pattern of change during starvation is shown in Fig. 9. In 1977 the levels tended to double from 25-30 to 50-60 mg/dl after 3 days, but by day 7 there was a large scatter in results. Uraemia was evident in the S-L group and in a more severe form in the NS-D group at the time of slaughter.

Thus uraemia could be considered to be a feature of the disease as it was recorded in S-L ewes but not in NS-L ewes. However some ewes in the NS-D group were uraemic but did not show symptoms.

2.1.7 CREATININE:

Creatinine levels also act as indicators of renal function. In both years the concentrations were stable in all groups up to day 5 of starvation (Fig. 10) and then increased.

In 1977, the NS-D group had significantly higher levels after seven days starvation. The S-L group was uraemic but did not have elevated creatinine levels, suggesting that uraemia was not due to

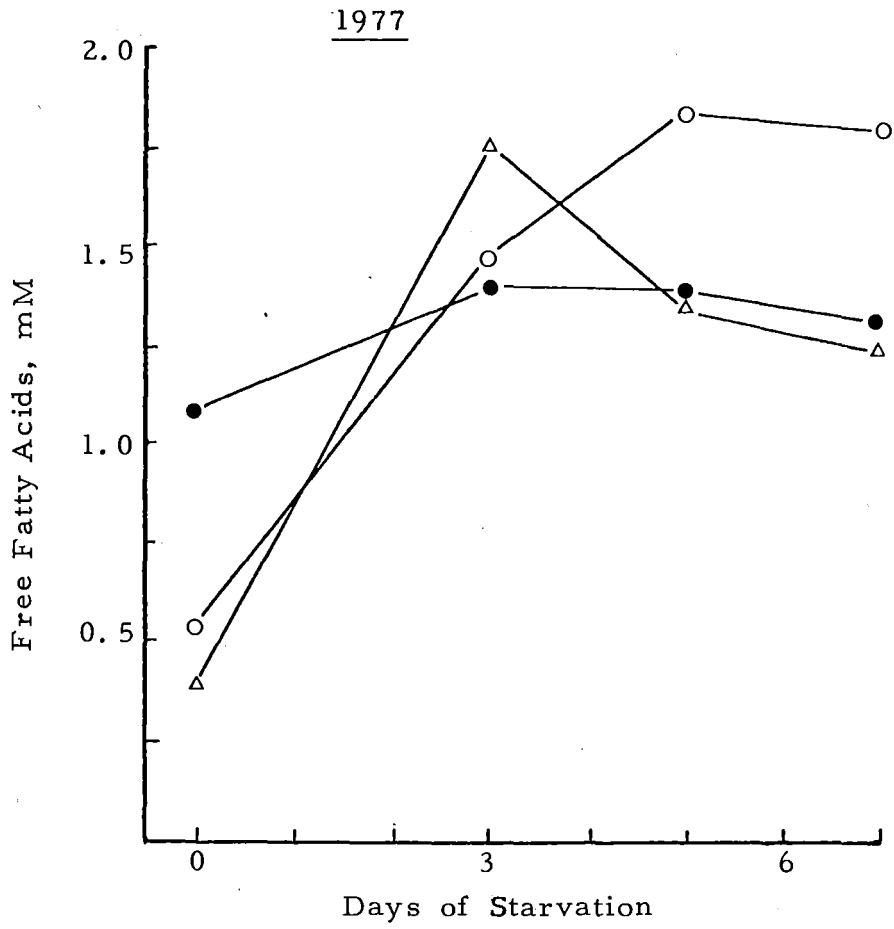


Fig. 8: Changes in plasma free fatty acids during starvation of twin-pregnant ewes. Ewes were susceptible (S) or non-susceptible (NS) to toxæmia, and at slaughter had live (L) or dead (D) lambs.

S-L (●)

NS-L (○)

NS-D (Δ)

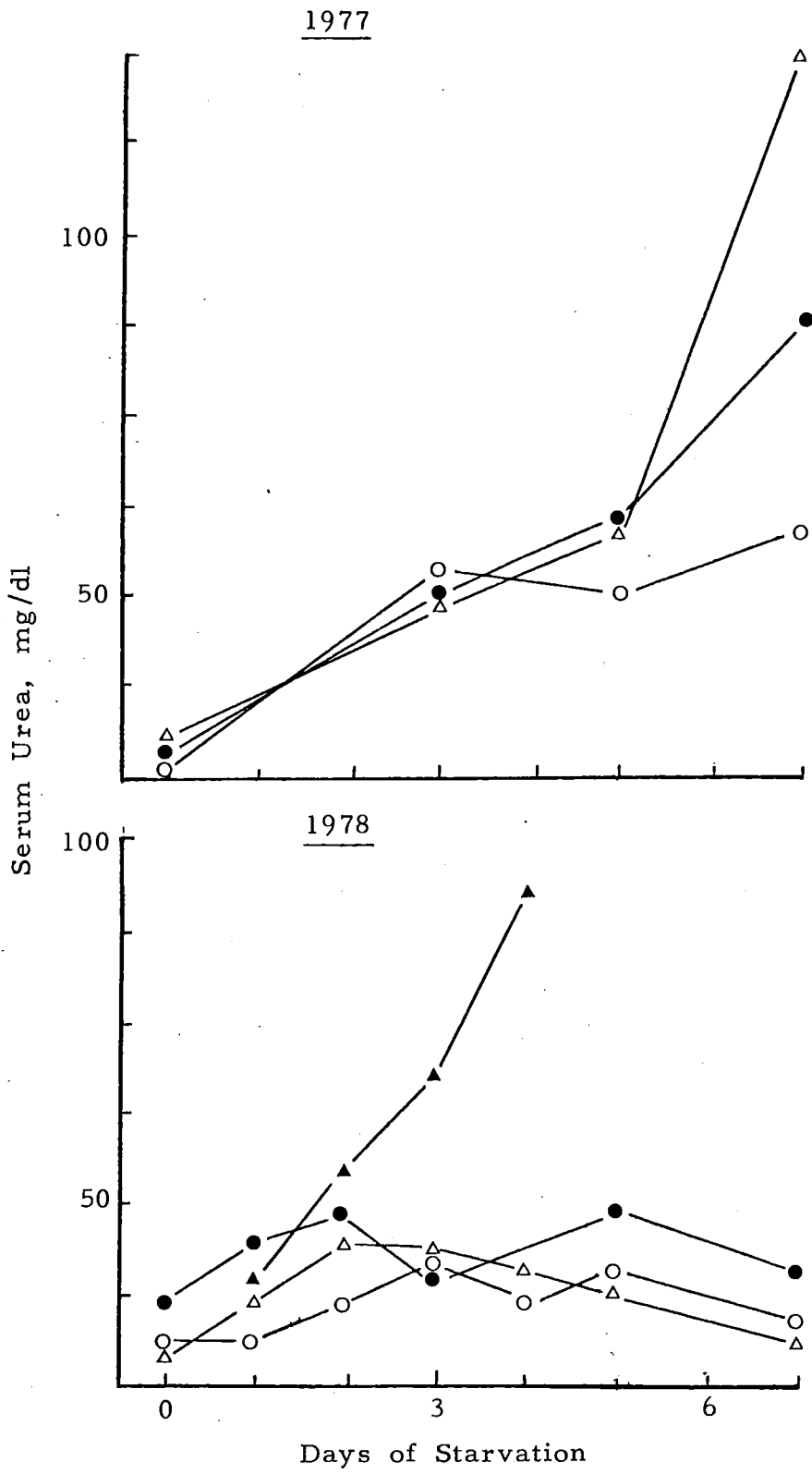


Fig. 9: Changes in serum urea during starvation of twin-pregnant ewes. Ewes were susceptible (S) or non-susceptible (NS) to toxaemia, and at slaughter had live (L) or dead (D) lambs.

S-L (●)	NS-L (○)
S-D (▲)	NS-D (△)

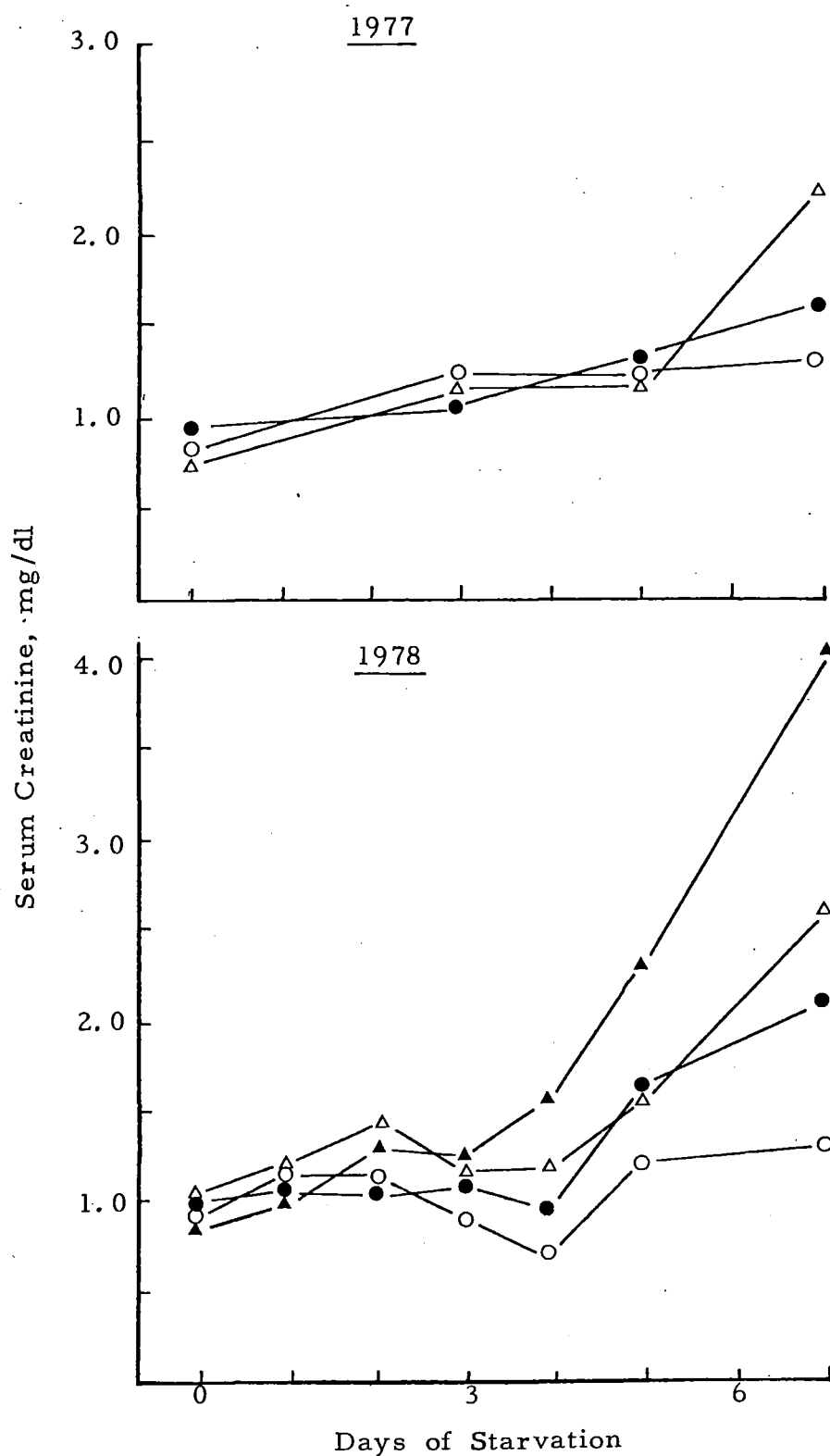


Fig 10: Changes in serum creatinine during starvation of twin-pregnant ewes. Ewes were susceptible (S) or non-susceptible (NS) to toxæmia, and at slaughter had live (L) or dead (D) lambs.

S-L (●)	NS-L (○)
S-D (▲)	NS-D (△)

renal failure. This is supported by the levels at time of slaughter. The S groups in 1978 had levels over 3 mg/dl, implying impaired renal function, while the NS-D group had very high levels which implied that many of the ewes in this group had renal failure.

Thus on starvation, creatinine levels increased but remained within the normal range, for NS-L ewes, and slightly above for S ewes. Concentrations in the NS-D group in both years were more variable, but indicated renal failure at the time of slaughter in some ewes.

These results would indicate that elevated levels are not associated with neurological symptoms of OPT.

2.1.8 INORGANIC PHOSPHATE:

Inorganic phosphate concentrations >9 mg/dl indicate renal failure.

Levels doubled in all groups over 7 days starvation, from pre-starve levels of 4 - 5 mg/dl (Fig. 11). The phosphate concentrations of the NS-L group rose more slowly than the other group after day 3; however at slaughter there was no difference between the NS-L and S-L groups. The values ranged from 9-11 mg/dl, indicating renal impairment in both groups, and supporting the view that renal damage was not necessarily associated with symptoms of OPT.

The highest concentration of inorganic phosphate, 17 mg/dl, was recorded in the NS-D group.

2.1.9 TOTAL CO_2

Total CO_2 is principally a measure of bicarbonate in the plasma and so indicates the buffer capacity of the blood or the acid-base status of the ewe. Levels fell throughout starvation (Fig. 11). The decline was similar in all groups over the first 3 days, but from

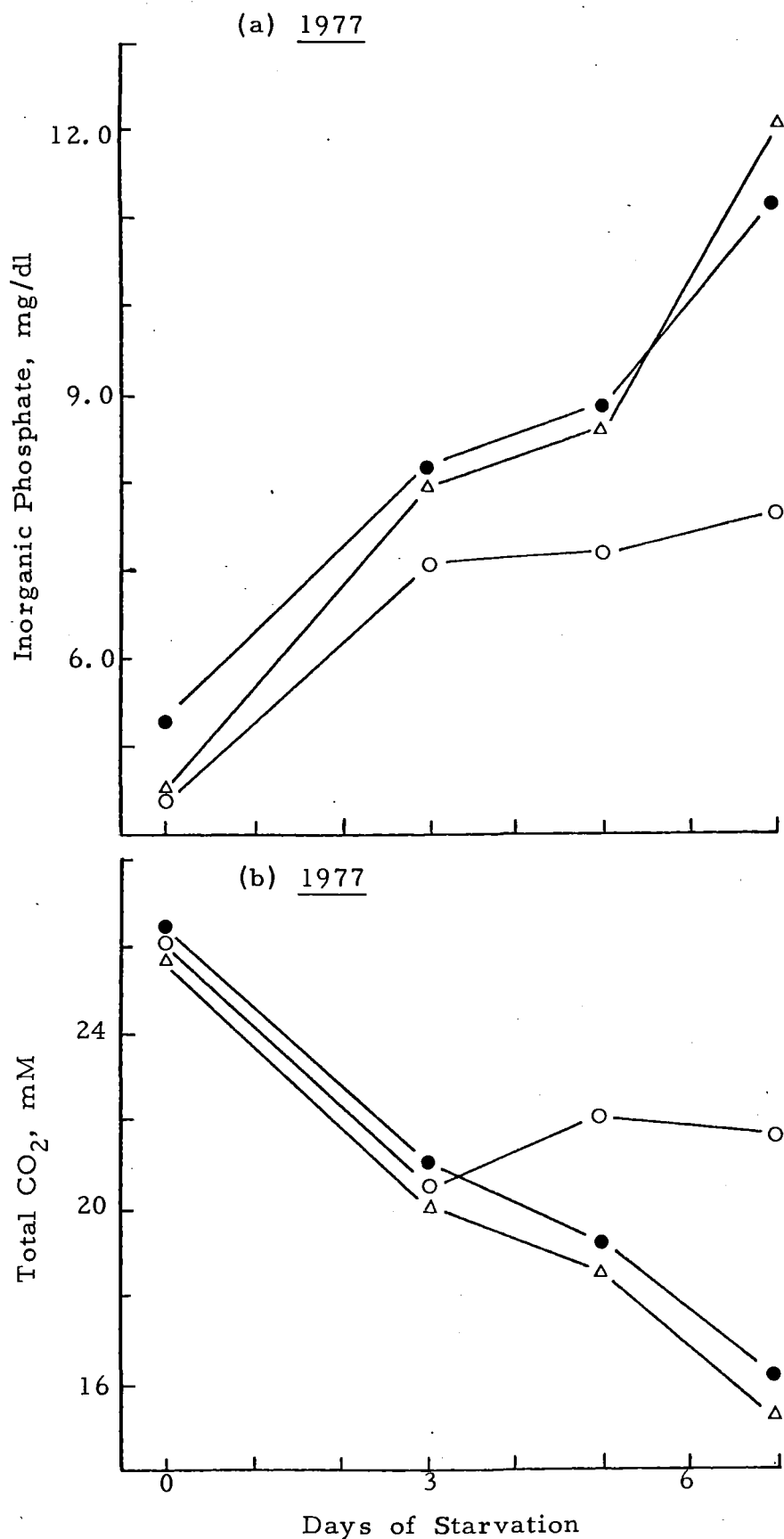


Fig. 11: Changes in serum (a) inorganic phosphate and (b) total CO₂ during starvation of twin-pregnant ewes. Ewes were susceptible to toxæmia with live lambs (S-L, ●) or non-susceptible with live (NS-L, ○) or dead (NS-D, Δ) lambs.

day 5 the NS-L group had significantly higher levels, although at the time of slaughter there was no difference between S-L and NS-L groups. The NS-D had significantly lower levels, which may be a consequence of the severe renal failure. The state of the lambs appeared to have had more effect on total CO₂ levels than the presence of OPT symptoms.

2.1.10 TOTAL PLASMA PROTEIN:

Levels were steady over the first 3 days of starvation, at about 6.7 g/dl, and then rose by about 12% (Fig. 12). There was no difference in the concentrations between groups throughout starvation or at the time of slaughter.

2.1.11 ALBUMIN:

Albumin changed in a similar way to total protein and the plasma levels had risen about 10% in all groups after 3 days of starvation. There was no significant difference between the groups during the starvation period, or at the time of slaughter (Fig. 12).

2.1.12 LIVER FAT:

In 1977 ewes with live lambs had higher fat levels (16-20% wet weight) than those with dead lambs (9% wet weight). There was no difference in fat levels between S and NS groups.

In 1978 the S group tended to have higher fat levels although symptoms of OPT were not always associated with high liver fat levels.

2.1.13 LIVER GLYCOGEN:

S ewes had significantly lower glycogen levels (2 mg/g) than NS-D (18 mg/g).

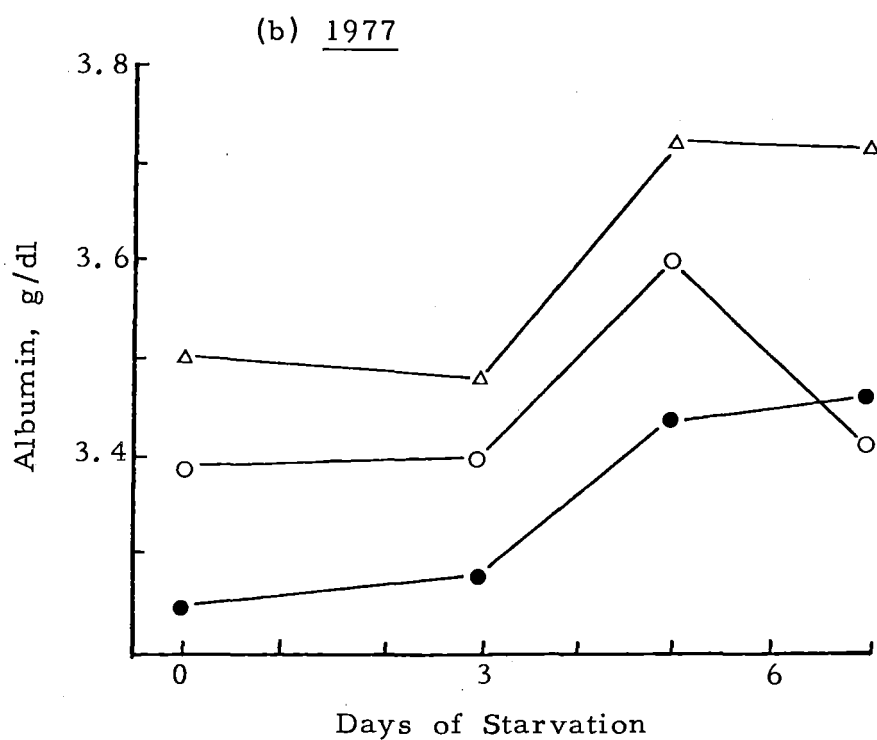
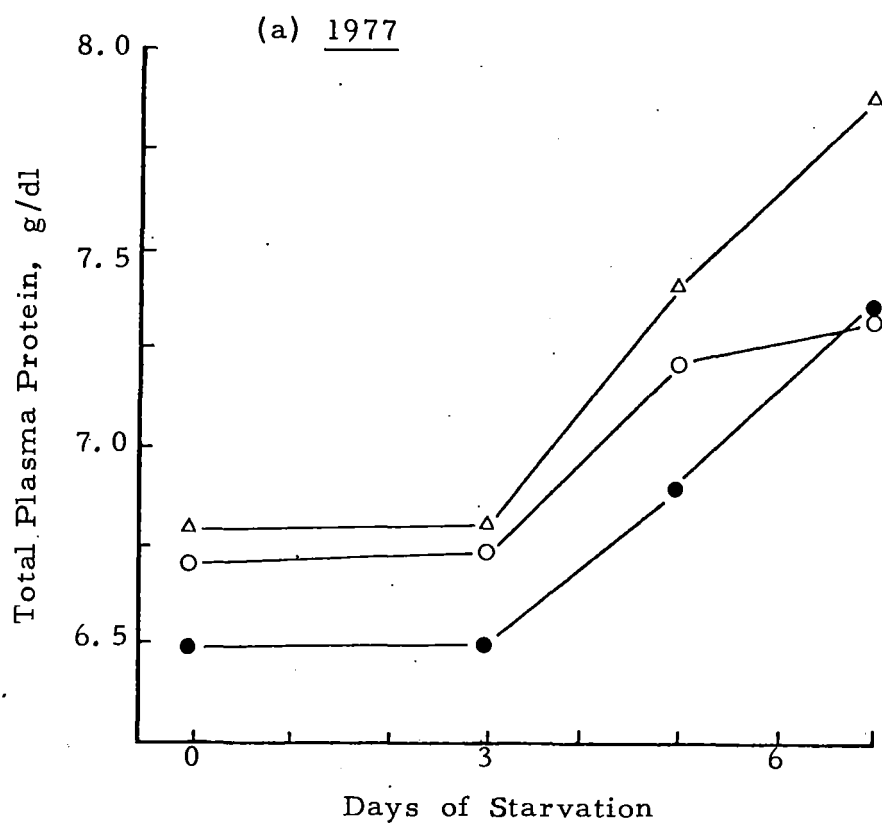


Fig. 12: Changes in (a) total plasma protein and (b) albumin during starvation of twin-pregnant ewes. Ewes were susceptible to toxæmia with live lambs (S-L, ●), or non-susceptible with live (NS-L, ○) or dead (NS-D, △) lambs.

2.2 NON-PREGNANT EWES:

Non-pregnant ewes fasted up to 10 days, did not have significantly different plasma glucose levels than those fasted 1-2 days (Table 6). No difference was found between the groups in liver glycogen, but those fasted more than 4 days had significantly higher liver fat (12 mg/g) than those fasted 1-2 days (7 mg/g).

2.3 SUMMARY:

Changes in blood parameters during starvation were similar for the NS and S ewes. The main changes were a fall in plasma glucose and total CO₂ and increases in ketone bodies, free fatty acids and inorganic phosphate. Creatinine and urea rose after 5 days, indicating impaired renal function.

The only differences in blood metabolites observed between S and NS ewes (with lambs in the same state) were the higher concentrations of total CO₂ on day 5, acetoacetate on days 3-5, free fatty acids on day 7, and the lower concentration of inorganic phosphate on day 7 in the NS group.

No metabolite differences could be shown between the groups at the time of slaughter, but on post-mortem S ewes had lower liver glycogen levels than NS-D ewes.

When non-pregnant ewes were starved 10 days, liver fat levels increased but no change occurred in plasma glucose or liver glycogen (Table 6).

3. ORGAN WEIGHTS OF SUSCEPTIBLE AND NON-SUSCEPTIBLE EWES.

The post-mortem data of various organs from starved pregnant ewes are listed in Table 7.

TABLE 6: Plasma Glucose and Liver Metabolites of Starved Non-Pregnant Ewes.

Starvation Period (Days)	No.	PLASMA GLUCOSE (mg/dl)		L I V E R	
		Prestarve	Slaughter	Fat (% wet weight)	Glycogen (mg/g)
1- 2	3	59 \pm 12	65 \pm 21	6.8 \pm 1.4 ^a	7.2 \pm 8.0
3- 4	3	61 \pm 6	76 \pm 11	11.6 \pm 2.8 ^b	11.6 \pm 8.9
9-10	3	72 \pm 11	72 \pm 13	13.1 \pm 2.7 ^b	11.7 \pm 2.4

Mean \pm S.D.

Means with different superscripts differ significantly at 5% level, based on the t-test.

TABLE 7: Organ Weights of Starved Pregnant Ewes.

1977

Group	No.	TOTAL FOETAL			EWE					
		Weight (kg)	Liver (g)	Adrenals (mg)	Weight (kg)	Liver (g)	Kidney (g)	Pancreas (g)	Adrenals (g)	Thyroid (g)
S-L	11	5.3 ± 0.8	126 ^a ± 23	592 ± 165	48 ^{ab} ± 6	822 ^{ab} ± 145	138 ^a ± 21	37 ± 13	7.1 ± 1.4	4.9 ^a ± 1.0
NS-L	8	5.4 ± 0.7	113 ^{ab} ± 15	786 ± 275	46 ^a ± 2	748 ^a ± 79	132 ^a ± 13	25 ± 16	7.1 ± 0.9	4.9 ^{ab} ± 1.8
NS-D	4	6.5 ± 1.4	187 ^b ± 74	618 ± 100	49 ^b ± 3	961 ^b ± 39	190 ^b ± 44	33 ± 15	8.9 ± 3.2	6.3 ^b ± 1.2

1978

Group	No.	TOTAL FOETAL	EWE		
		Weight (kg)	Weight (kg)	Liver (g)	Adrenals (g)
S-L	4	4.6 ± 0.7	39 ± 4	726 ± 143	7.1 ± 1.3
S-D	9	4.8 ± 0.8	39 ± 5	746 ± 138	9.2 ± 2.1
NS-L	1	4.4	47	721	6.0
NS-D	6	5.4 ± 0.8	44 ± 8	807 ± 79	7.3 ± 1.0

Mean ± S.D.

Means with different superscripts differ significantly at 5% level, based on the t-test

Ewes were susceptible (S) or non-susceptible (NS) to pregnancy toxæmia, with live (L) or dead (D) lambs in utero at slaughter.

3.1 FOETAL DATA:

The total foetal weight was calculated for each group. There was no difference between the S-L or NS-L groups, but in both years the NS-D group had heavier foetuses.

The foetal liver weight of NS-D was significantly higher than S-L but was a feature of the foetal weight.

Although the difference was not significant, NS-L had higher foetal adrenal weights, which, when divided by foetal weight, were 50% higher than the S-L group. This may indicate foetuses in the NS-L group were responding to stress.

3.2 MATERNAL DATA:

In 1977 ewes in the NS-L group had significantly lower live-weights than NS-D and could indicate greater mobilization of body reserves, or might reflect the different foetal weights. In 1978 the S groups tended to be lighter.

The NS-D group had larger livers and thyroids which may be related to their having larger foetuses.

The kidneys of the NS-D group were significantly heavier. As swelling was often noticed and kidney failure had occurred in this group, the increased weight could be due to hypertrophy or to kidney damage.

No significant difference was detected between pancreas weights or adrenal weights, although the NS-D group had adrenals 20% larger than the other two groups. This hypertrophy may indicate an increased response to stress; however the observation was not repeated in 1978 when S-D ewes had enlarged adrenals.

4. GLUCOSE TOLERANCE AND INSULIN RESISTANCE OF FED SHEEP.

4.1 RELIABILITY OF GLUCOSE TOLERANCE ($T_{\frac{1}{2}}$) ESTIMATION:

In Fig. 13, a plot is shown of the first estimate of $T_{\frac{1}{2}}$, against the second estimate. The 95% confidence interval for one estimation is ± 6.7 min.

4.2 GLUCOSE TOLERANCE ($T_{\frac{1}{2}}$), EXTRAVASCULAR INSULIN (I') AND INSULIN RESISTANCE (R):

Preinjection plasma glucose concentrations (Table 8) were similar in pregnant and non-pregnant sheep. Insulin levels of the pregnant sheep fell within a narrow range and were similar to the non-pregnant ewes on the same feeding regime; however the levels were higher in ewes fed once daily.

The individual animal values are shown in Appendix 5, and are summarized in Table 8. The glucose tolerance of ewes later shown to be susceptible to OPT was significantly higher (50 min) than NS group with live lambs (38 min). Thus the NS-L group was able to use glucose more rapidly. There was no difference in $T_{\frac{1}{2}}$ between S-L and NS-D groups.

The mean extravascular insulin levels, when plasma glucose was 100 mg/dl above basal, were similar for all groups and ranged from 26-41 μ U/ml. However there was considerable variability between animals.

The insulin resistance of the S group was significantly higher (2043 μ U/ml.min) than that of the NS-L group (1261 μ U/ml.min) and also higher than the NS-D group (1055 μ U/ml.min).

A high resistance value arises from a high insulin production but slow glucose disappearance, and a curve of a S-L ewe is shown

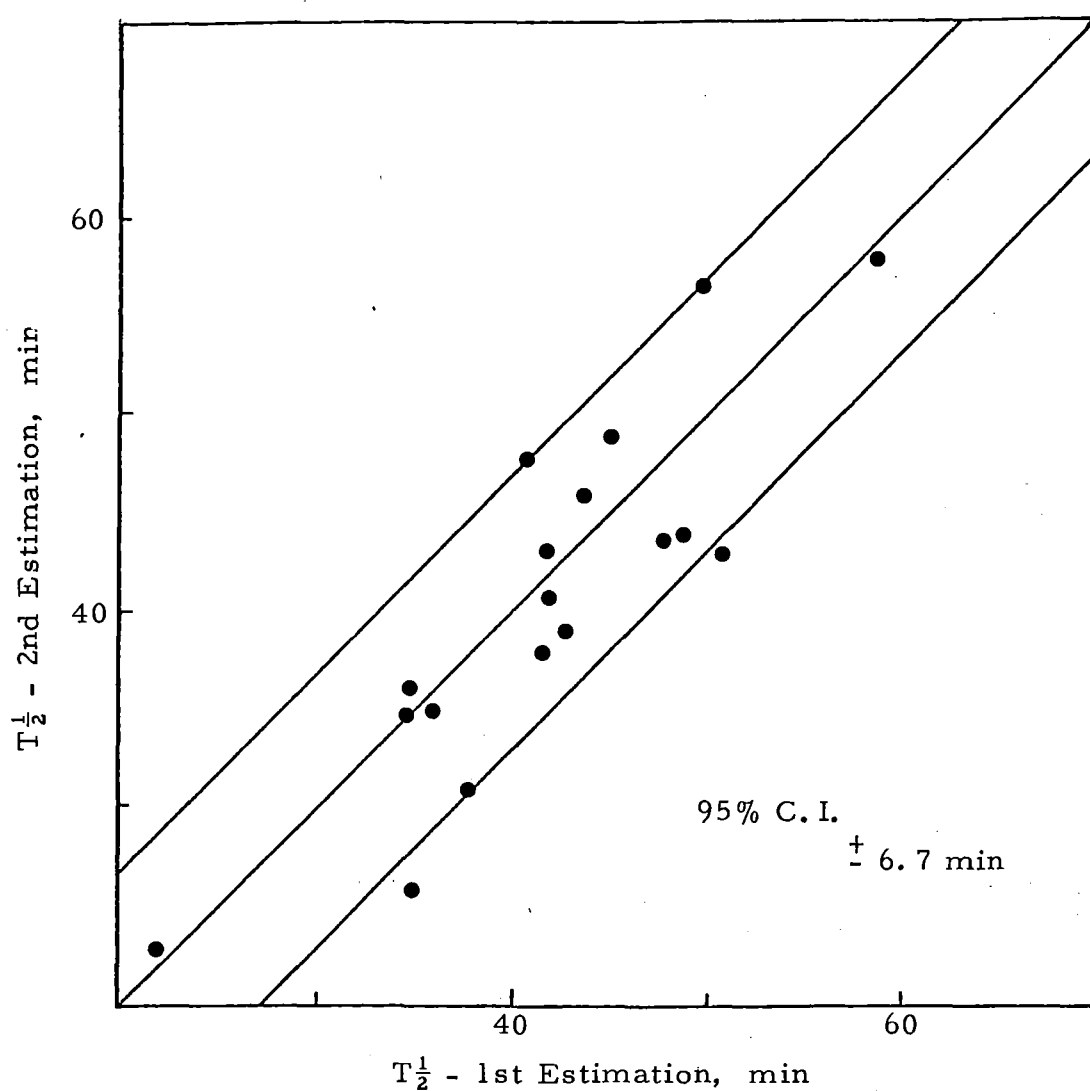


Fig. 13: Glucose tolerance tests - 95% confidence interval around the mean of two estimates of the half-life of glucose ($T_{\frac{1}{2}}$), in twin-pregnant ewes.

TABLE 8: Glucose Tolerance and Insulin Resistance of Ewes.

Group	No.	Pre-Injection		Post-Injection		
		Glucose (mg/dl)	Insulin (μ U/ml)	$T_{\frac{1}{2}}$ (min)	I' (μ U/ml)	R (μ U/ml. min)
a) Twin-Pregnant						
Susceptible Live	7	60 ± 8	18 ± 5	50 ± 10^a	41 ± 10	2043 ± 670^a
Non-susceptible Live	6	58 ± 7	16 ± 9	38 ± 10^b	35 ± 12	1261 ± 433^b
Dead	3	52 ± 12	11 ± 8	52 ± 23^{ab}	26 ± 23	1055 ± 690^{ab}
b) Non-Pregnant						
Fed Daily	2	60	31	40 ± 22	86 ± 4	3186 ± 1478
Fed Hourly	2	60	10	36 ± 4	41 ± 15	1394 ± 407

Mean \pm S. D.

Means with different superscripts differ significantly at 5% level, based on t-test.

Plasma glucose and insulin concentrations were measured for two hours after an intravenous injection of glucose (0.4 g/kg), and glucose tolerance ($T_{\frac{1}{2}}$), extravascular insulin concentration, when plasma glucose was 100 mg/dl above basal (I') and insulin resistance (R) were determined.

The pregnant ewes were classed after a period of starvation as susceptible or non-susceptible to pregnancy toxæmia with live or dead lambs in utero at slaughter.

in Fig. 14a. Low resistance, as shown by the NS groups, can arise from a high insulin production, and fast glucose disappearance (see Fig. 14b) or, a low insulin production and slow disappearance rate (Fig. 14c).

Thus, by measuring $T_{\frac{1}{2}}$, I' and calculating insulin resistance it could be possible to predict how ewes would react to starvation. Ewes with long $T_{\frac{1}{2}}$ and high resistance appear to be susceptible to OPT, while ewes with low resistance appear to be non-susceptible.

Results of experiments on non-pregnant ewes are also included in Table 8. The ewes were either fed daily or hourly. There was no difference in the $T_{\frac{1}{2}}$ values; however the ewes fed once daily had mean I' levels of 86 $\mu\text{U/ml}$ which were twice as high as those fed hourly (41 $\mu\text{U/ml}$). This was significant at the 6% level based on the t-test. The sheep fed daily thus had higher resistance, which means when challenged with a glucose load they had to produce more insulin to achieve the same rate of glucose uptake.

As the values of $T_{\frac{1}{2}}$, I' and R , of the sheep fed hourly closely approximate the values of NS-L ewes (also fed hourly) insulin resistance would not seem to occur during pregnancy in sheep that are non-susceptible to OPT.

5. GLUCOSE KINETICS OF FED, PREGNANT EWES.

5.1 ALL SHEEP:

5.1.1 FITTED CURVES

The specific activity values for all sheep were averaged at each sampling time, by taking the geometric mean. The averaged values are shown in Table 9. Consistent differences occurred between the ^3H and ^{14}C tracers after 90 min, and can be seen graphically in the fitted decay curves in Fig. 15. The parameters of these fitted curves

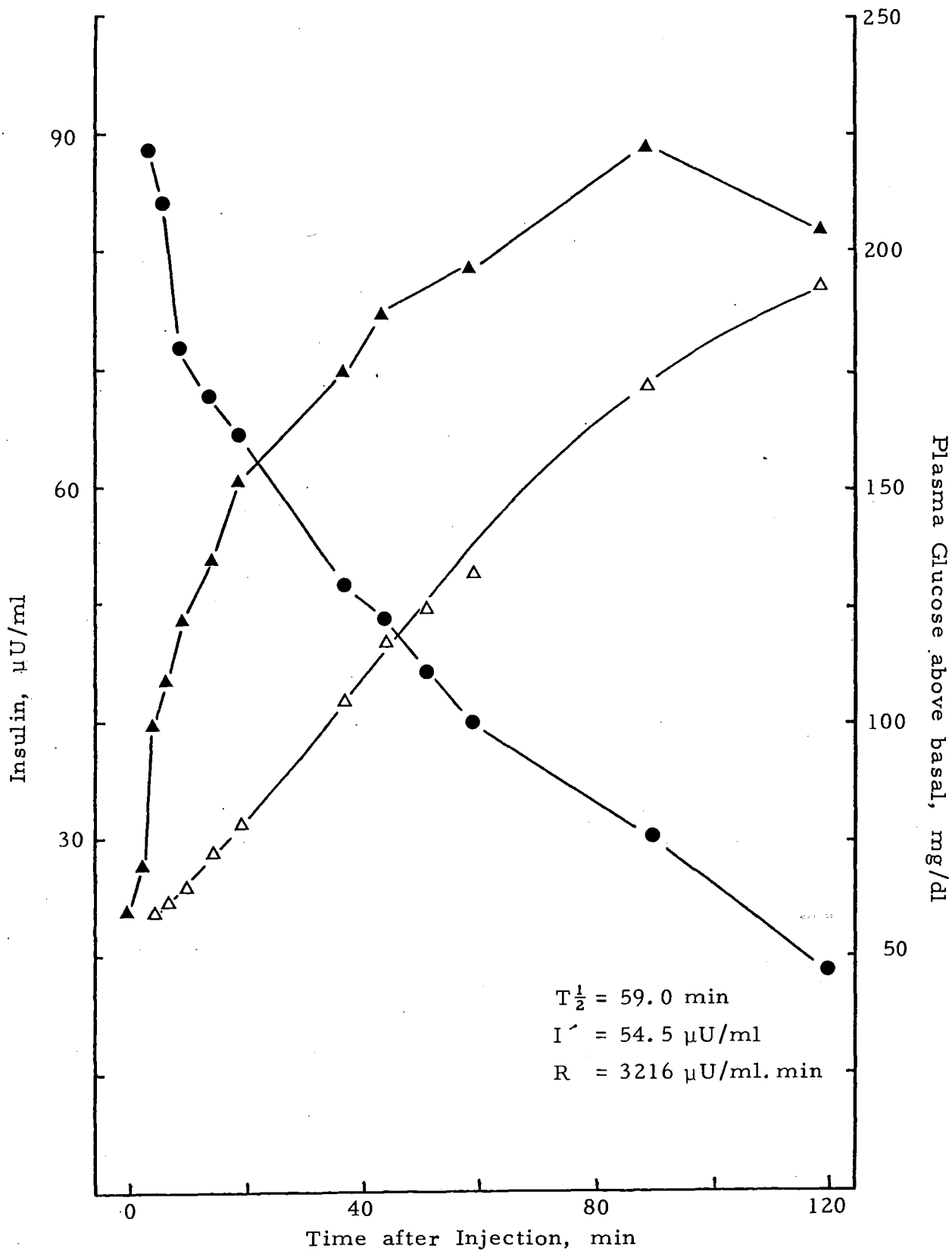
Fig. 14: Changes in glucose (●), insulin (▲) and extravascular insulin (Δ), after the injection of glucose (0.4 g/kg) into three twin-pregnant ewes.

$T_{\frac{1}{2}}$ - glucose tolerance

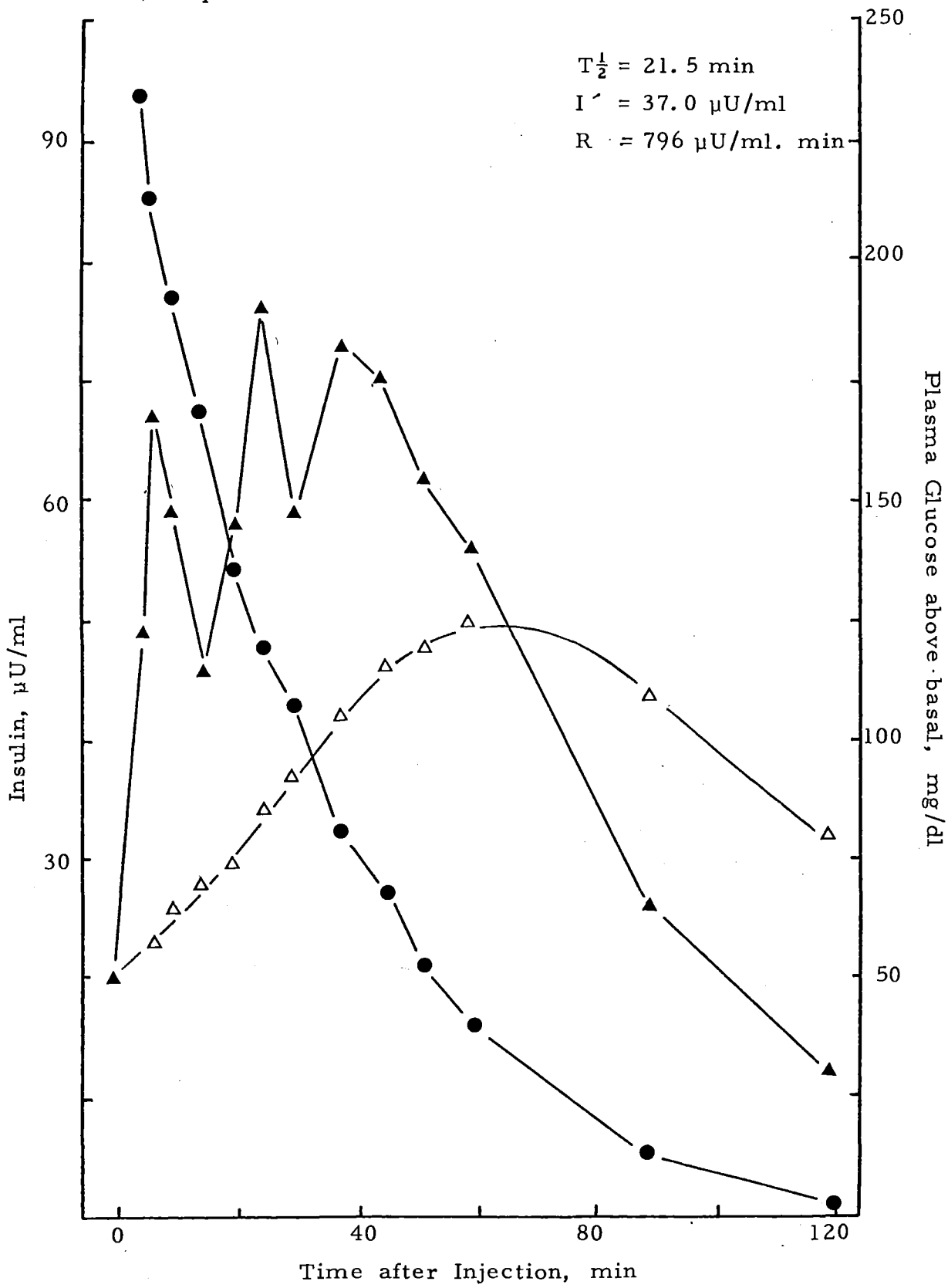
I' - extravascular insulin when glucose is 100 mg/dl
above basal

R - insulin resistance index.

a) Expt. 17



b) Expt. 38



c) Expt. 9.

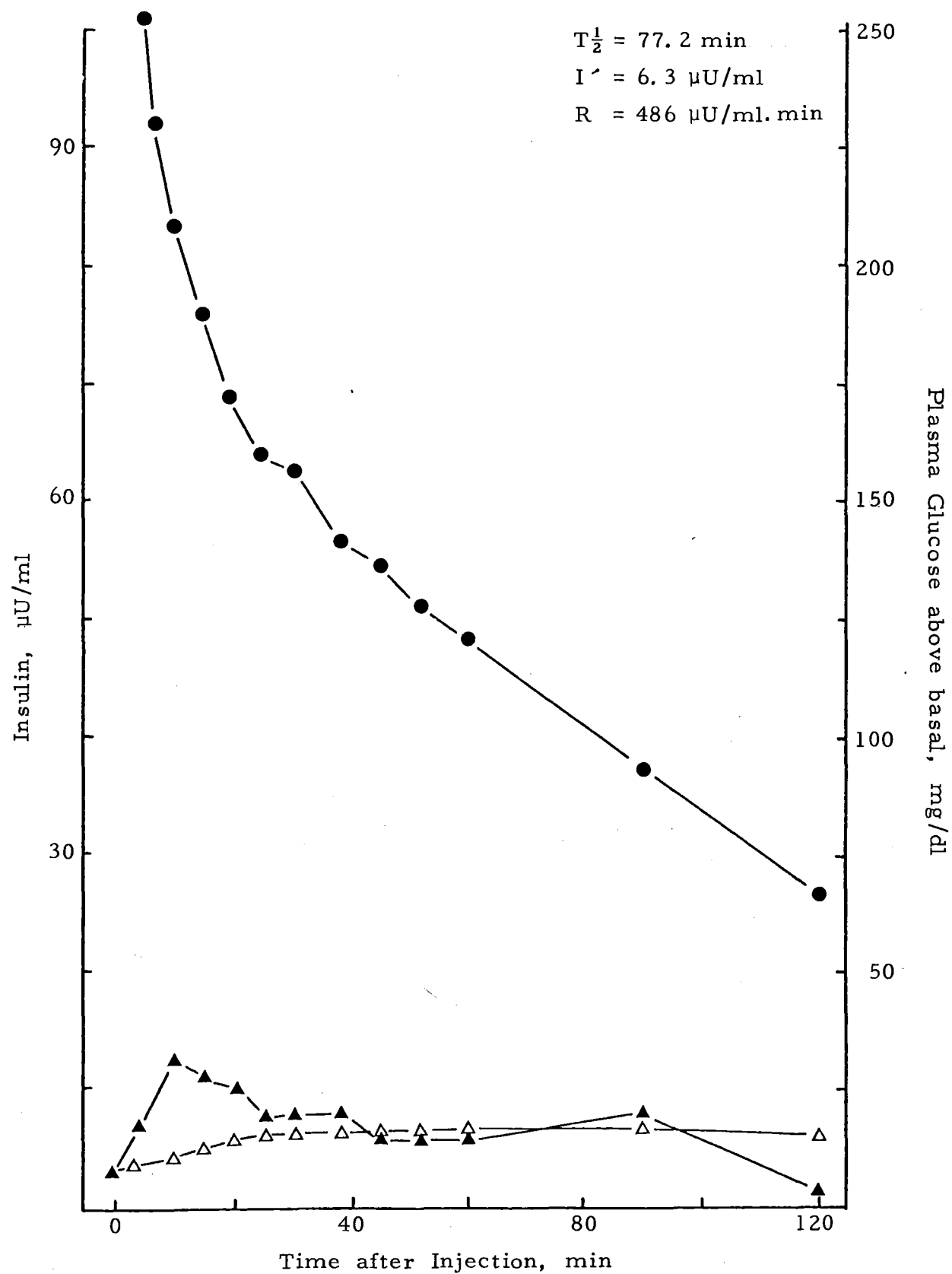


TABLE 9: Tracer Kinetics - Observed Data
(15 Twin Pregnant Sheep)

Time (min)	Specific Activity (fraction of dose/mg glucose)	
	^{14}C	^3H
0.72	0.30706E-03	0.31783E-03
1.07	0.26613E-03	0.27284E-03
1.56	0.24710E-03	0.24769E-03
2.07	0.22404E-03	0.23515E-03
3.00	0.20879E-03	0.21221E-03
5.10	0.18114E-03	0.18430E-03
7.09	0.16164E-03	0.16775E-03
10.0	0.14460E-03	0.14731E-03
14.0	0.12219E-03	0.12760E-03
20.2	0.10936E-03	0.11300E-03
30.0	0.90944E-04	0.91779E-04
40.2	0.73862E-04	0.76042E-04
60.1	0.52395E-04	0.52984E-04
89.8	0.35921E-04	0.34130E-04
120.2	0.23738E-04	0.21359E-04
181.1	0.11561E-04	0.93754E-05
303.3	0.34574E-05	0.21201E-05
420.5	0.14537E-05	0.72594E-06
540.2	0.76845E-06	0.30681E-06
720.0	0.43448E-06	0.16620E-06
1414.0	0.21908E-06	0.88441E-07
2170.0	0.13212E-06	0.38904E-07
2800.0	0.15011E-06	0.51582E-07

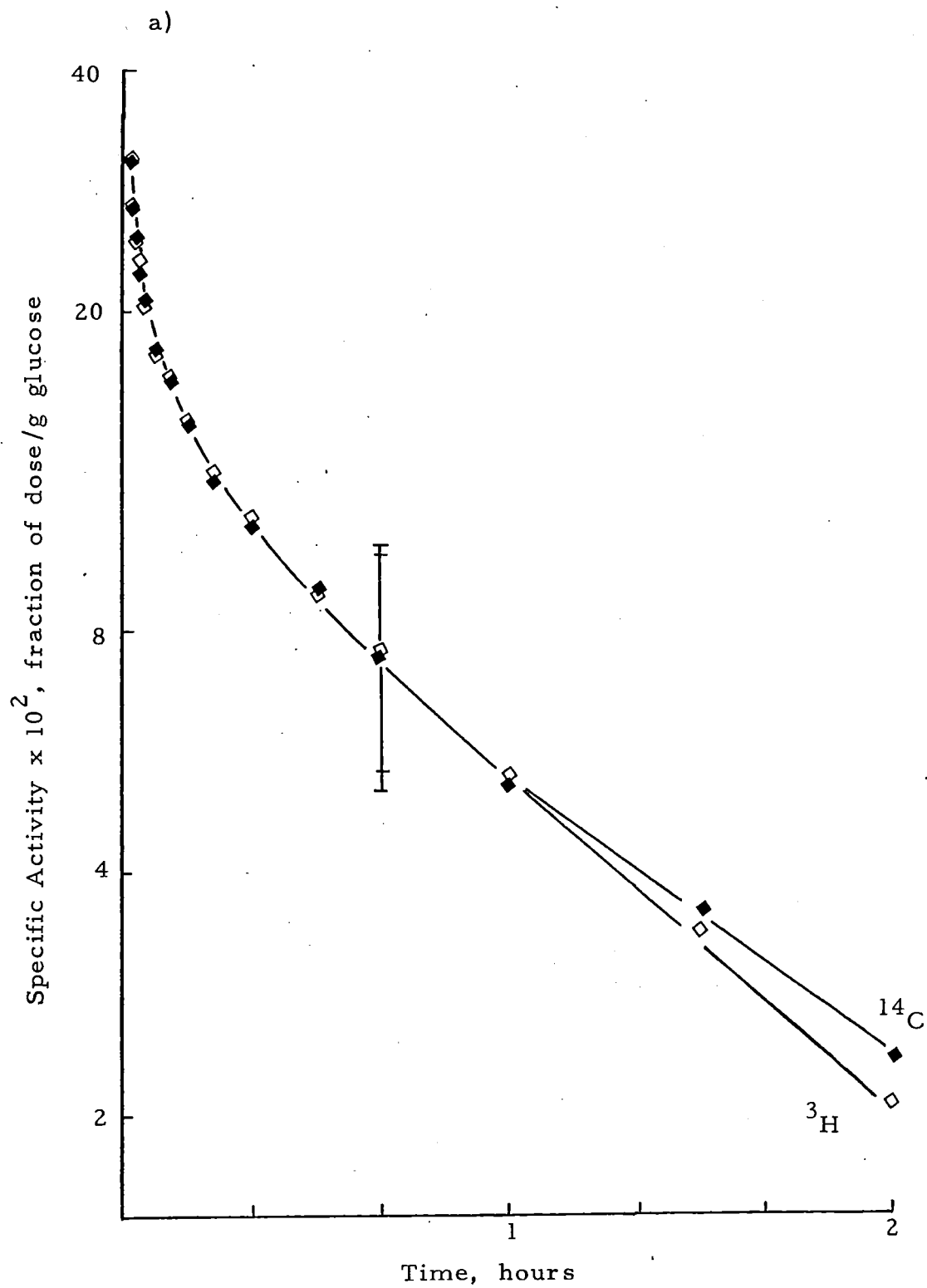
Plasma glucose specific activity was measured, in each ewe, following the injection of 0.3 mCi [$\text{U-}^{14}\text{C}$] and 1 mCi [$6\text{-}^3\text{H}$] glucose.

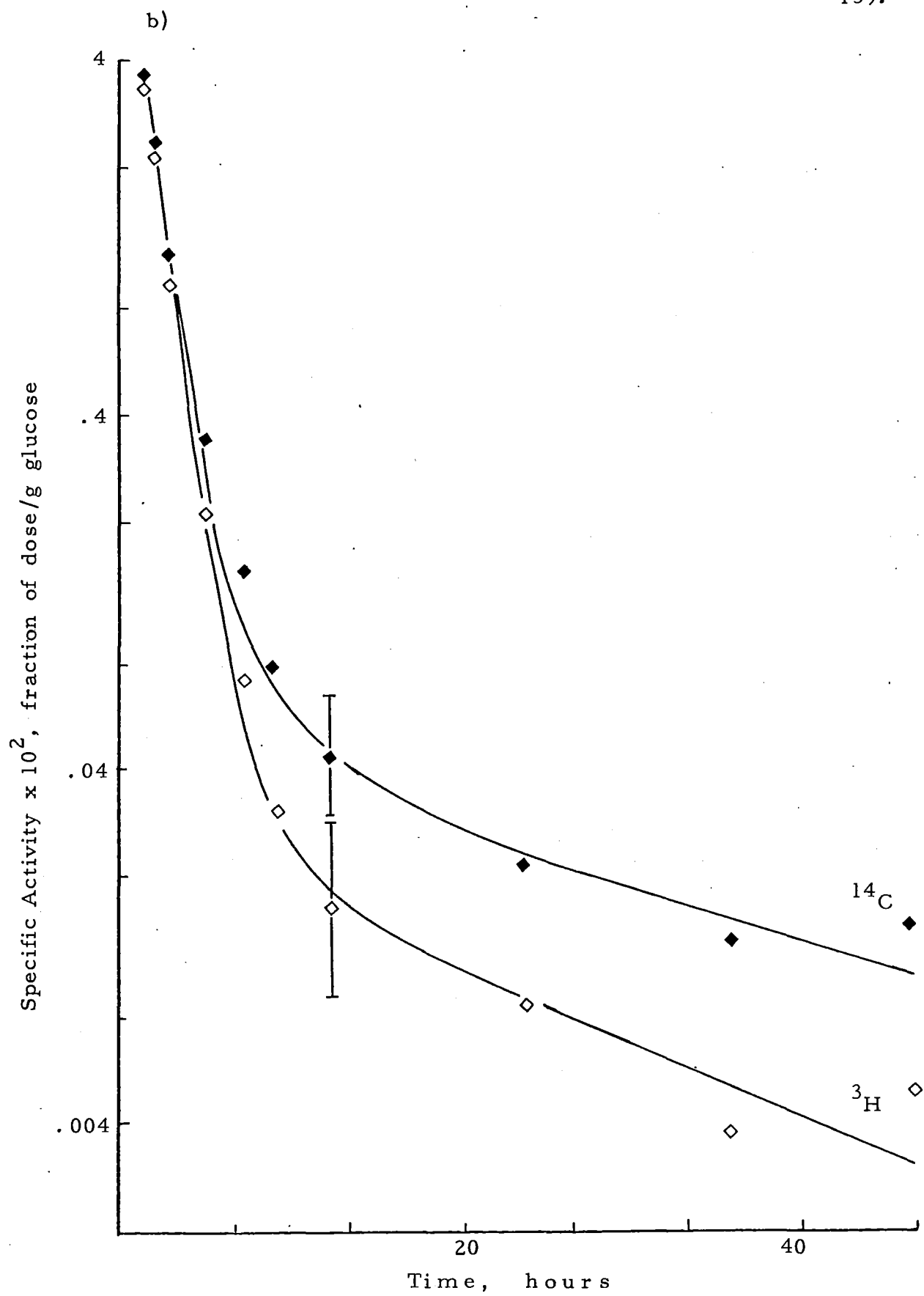
Fig. 15: Plasma glucose specific activity following the injection of $[U-^{14}C]$, $[6-^3H]$ glucose into twin-pregnant ewes.

The points are the geometric means of observations on 15 sheep and the curves represent the fitted sum of five exponentials.

The bar shows a 95% confidence interval about the data.

- a) Disappearance over two hours after the tracer injection
- b) Disappearance over 48 hours after the tracer injection.





are given in Table 10. The data were best fitted by an equation with five exponential terms, as with five terms the consistent deviations between the observed and predicted values which occurred with four terms, were absent, and the sum of squares was reduced (Table 11). No improvement in fit was obtained with six terms.

When glucose turnover was calculated, as the reciprocal of area under the sum of exponentials curve (area is the sum, of intercepts divided by the slopes) the value for ^{14}C was 125 g/day and for ^3H , 138 g/day. This represents 9.4% recycling if all the tritium is lost from the system.

5.1.2 MODEL A:

When the L's (slopes) were mapped to a 5-compartment series model (Model A, Fig. 16) and solved, parameters for the fractional flow rates were obtained and these are shown in Table 12. The steady state solution for movement of tracee is shown in Fig. 17. The physiological interpretation of this model is set out in Table 13. The first three compartments were of similar size with both tracers and implied that movement of [^{14}C] and [^3H] glucose was similar between these compartments. The size of the first compartment was about that of plasma, when expressed as plasma equivalent volume, or % body weight, and the sum of the first three compartments was approximately equal to extracellular fluid volume. Thus compartment 1 appeared to be plasma and compartments 2 and 3 interstitial fluid.

Compartment 5, based on its size, would not seem to be just glucose, and was possibly products and precursors of glucose.

The turnover time of each compartment is shown in Table 12. The first two compartments turned over very rapidly and could be influenced by circulation time. However Hevesy and Jacobsen (1940)

TABLE 10: Fitted Curve (15 Twin Pregnant Sheep)

$$F_1 = S(1, 2)e^{-L(0, 2)^t} + S(1, 3)e^{-L(0, 3)^t} + S(1, 4)e^{-L(0, 4)^t} + S(1, 5)e^{-L(0, 5)^t} + S(1, 6)e^{-L(0, 6)^t}$$

Intercept	¹⁴ C	³ H
S(1, 2)	0.29531E-03 (31) ⁺	0.44581E-03 (30)
S(1, 3)	0.11538E-03 (5)	0.12419E-03 (3)
S(1, 4)	0.88446E-04 (13)	0.10182E-03 (25)
S(1, 5)	0.68994E-04 (22)	0.28848E-06 (51)
S(1, 6)	0.63793E-06 (33)	0.56246E-04 (51)
Slope		
L(0, 2)	0.24095E+01 (21)	0.29061E+01 (16)
L(0, 3)	0.21762E+00 (13)	0.21492E+00 (8)
L(0, 4)	0.28318E-01 (18)	0.22576E-01 (12)
L(0, 5)	0.10311E-01 (9)	0.78588E-03 (47)
L(0, 6)	0.65110E-03 (36)	0.11446E-01 (19)

⁺ % Fraction Standard Deviation of each parameter is shown in brackets.

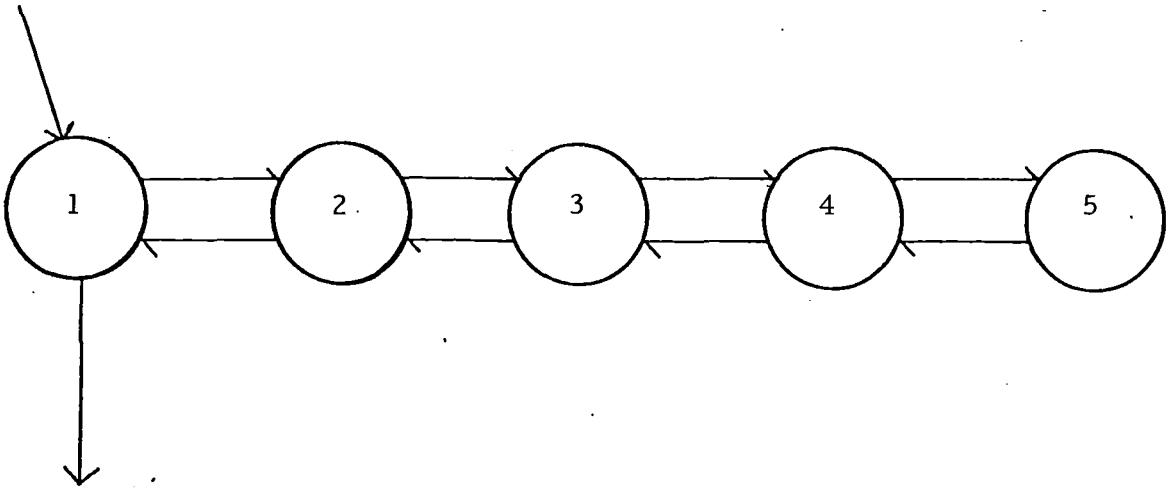
The parameters of a sum of exponentials curve, that describes the change in glucose specific activity following the injection of 0.3 mCi [U-¹⁴C]-and 1 mCi [6-³H] glucose.

TABLE 11: Improvement in Sum of Squares with Five Exponentials (15 Sheep)

Exponentials	Sum of Squares of Deviations	
	^{14}C	^3H
4	0.1546E-11	0.3415E-12
5	0.5255E-12	0.8372E-13
% Improvement	66.0	75.5

The sum of exponentials curve describes the change in plasma glucose specific activity following the single injection of 0.3 mCi [U- ^{14}C]-and 1 mCi [6- ^3H] glucose into twin-pregnant ewes.

MODEL A: Series Model



MODEL B: Model Incorporating a Recycling Flow.

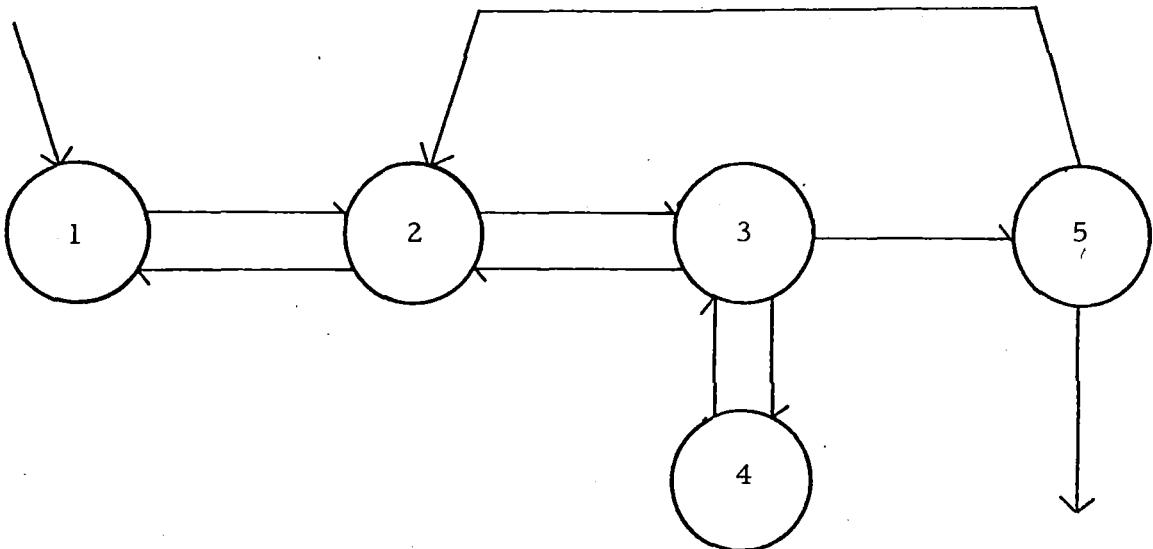


Fig. 16: Compartmental models.

TABLE 12: Fractional Flow Rates (L's) and Turnover Times -
Model A (All Sheep)

	¹⁴ C	³ H
L(0, 1)	0.049	0.071
L(2, 1)	1.23	1.84
L(1, 2)	1.06	1.07
L(3, 2)	0.135	0.122
L(2, 3)	0.130	0.148
L(4, 3)	0.014	0.011
L(3, 4)	0.015	0.020
L(5, 4)	0.00440	0.0031
L(4, 5)	0.00095	0.0010
L(1, 1)	1.28	0.91
L(2, 2)	1.20	1.19
L(3, 3)	0.144	0.159
L(4, 4)	0.019	0.023
L(5, 5)	0.00095	0.0010
Turnover Time (min)		
Compartment	¹⁴ C	³ H
1	0.78	0.52
2	0.83	0.84
3	6.94	6.29
4	52.6	43.5
5	1052.6	1000.0

The parameters apply to a 5-compartment series model which predicts the glucose kinetics of twin-pregnant ewes.

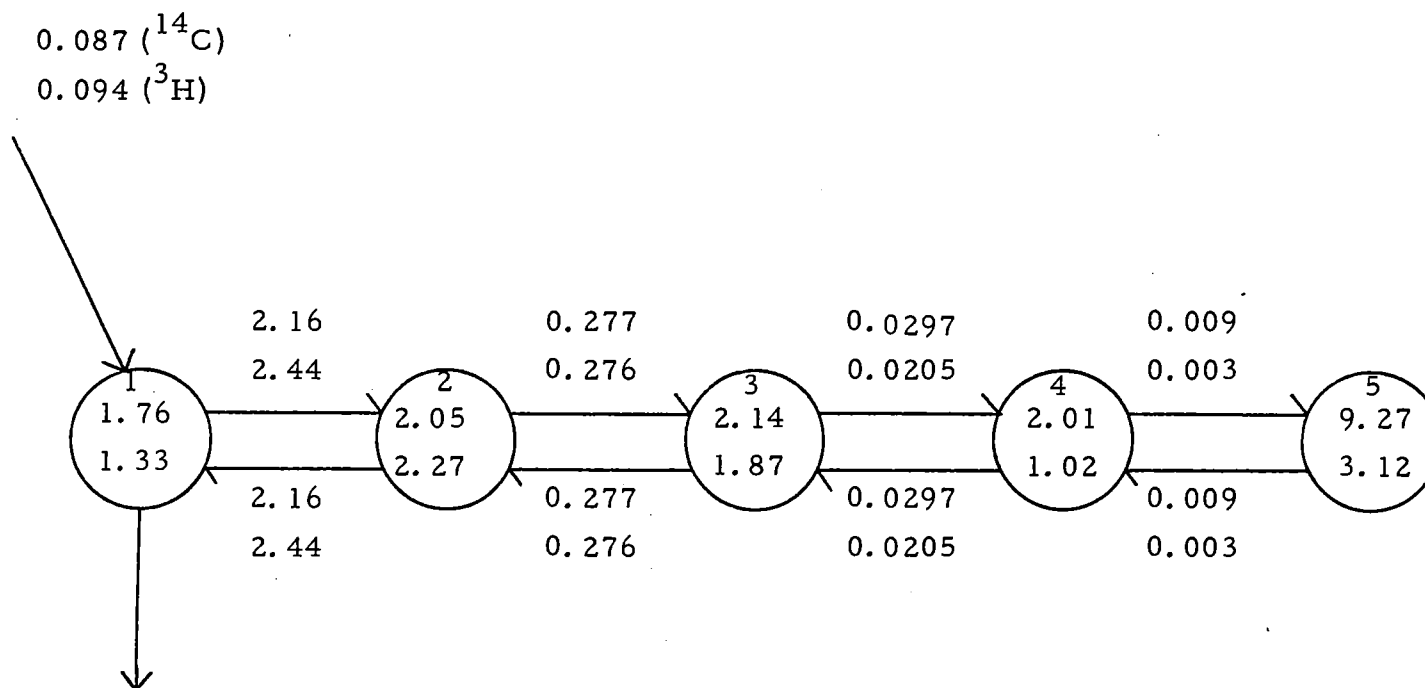


Fig. 17: Steady state solution for Model A.

Compartment size of glucose (g) and flow rates between compartments (g/min) are shown for each tracer. The steady state solution for this model is for twin-pregnant sheep.

TABLE 13: Physiological Interpretation - Model A

Average liveweight = 48 kg
Plasma glucose 65 mg/dl.
Plasma equivalent volume (PEV) = $\frac{\text{pool size (g)}}{\text{plasma glucose (g/l)}}$ litre

Compartment	Compartment Sizes			
	¹⁴ C		³ H	
	PEV (l)	LW (%)	PEV (l)	LW (%)
1	2.70	5.6	2.0	4.3
2	3.15	6.6	3.5	7.3
3	3.29	6.9	2.9	6.0
4	3.09	6.4	1.6	3.3
5	14.3	29.8	4.8	10.0

Body Fluid	Body Fluid Volume	
	Vol (l)	LW (%)
1. Extracellular fluid (ECF)		
Plasma	2.7	5
Interstitial Fluid	7.2	15
	9.6	20
2. Intracellular Fluid (ICF)	24	50

The steady state values apply to a 5-compartment series model which describes glucose kinetics in twin-pregnant ewes.

calculated that it took 30 sec for D_2O to move from plasma to the interstitial space.

5.1.3 MODEL B:

Glucose is recycled when it is catabolized to C_3 compounds then resynthesized into glucose by the liver cells. A recycling flow was introduced into the model from compartment 5 to compartment 2, as it was considered that glucose would be released into the interstitial fluid before moving into plasma (Fig. 16).

Loss was considered to occur from compartment 5, i.e. glucose would be metabolized and lost as $^{14}CO_2$ or 3H_2O , and so a flow rate $L(0, 5)$ was introduced to represent this.

Compartment 4 may have represented a foeto-placental compartment. Uterine uptake of glucose has been calculated as 0.048 g/min (Simmons et al., 1975). This roughly corresponded to $R(4, 3)$ (Table 14).

Limitations were imposed on the model as only one compartment (plasma) was sampled. Thus only 9 flow rates could be uniquely determined.

The fitted parameter values and steady state solution for each tracer fitted to Model B are shown in Table 14.

The main differences between the tracers were the amount that flowed back to compartment 2, $R(2, 5)$, and the loss from compartment 5, $R(0, 5)$. ^{14}C flowed back at twice the rate of 3H , and agrees with the findings of Kronfeld (1977) in cows, that some 3H does seem to recycle. For carbon, 8% of the amount lost as $R(0, 5)$ was recycled, and for 3H this value was 3%.

TABLE 14: Steady State Solution: Pregnant Sheep - Model B.

	^{14}C	^3H
Fractional Flow Rate (min^{-1})		
L(2, 1)	1.30	2.54
L(1, 2)	1.03	1.01
L(3, 2)	0.177	0.160
L(2, 3)	0.103	0.124
L(4, 3)	0.012	0.011
L(3, 4)	0.021	0.027
L(5, 3)	0.034	0.039
L(2, 5)	0.00006	0.00004
L(0, 5)	0.00069	0.00103
K(1)	0.00056	0.00096
Pool Size (g)		
Q_1	1.76	1.04
Q_2	2.13	2.54
Q_3	2.75	2.49
Q_4	1.54	1.01
Q_5	125.7	91.1
Flow Rate (g/min)		
R(2, 1)	2.28	2.65
R(1, 2)	2.19	2.55
R(3, 2)	0.377	0.41
R(2, 3)	0.284	0.31
R(4, 3)	0.033	0.027
R(3, 4)	0.033	0.027
R(5, 3)	0.094	0.097
R(2, 5)	0.0073	0.0032
R(0, 5)	0.0862	0.0940
U(1)	0.0862	0.0940

The steady state values apply to a 5-compartment model, which incorporates a recycling flow and describes glucose kinetics in twin-pregnant ewes.

5.2 GROUP DATA: SUSCEPTIBLE AND NON-SUSCEPTIBLE EWES:

5.2.1 FITTED CURVES:

Curves were fitted to each group of data separately, i. e. S-L, NS-L and NS-D and are drawn in Fig. 18. The parameters of these curves are listed in Table 15.

The NS-D group appeared to differ in the rate at which tracer was lost from the last compartment.

5.2.2 MODEL B:

The solution for each tracer was calculated for Model B and the fractional flow rates for each tracer are shown in Fig. 19.

5.2.1.1 Differences between tracers within each group:

To compare the differences, the $^{14}\text{C}/^3\text{H}$ ratio was calculated for each flow rate for each group, and are shown in Table 16.

The main difference between tracers in all groups were, the flows L(2, 5), in which ^{14}C was at least twice ^3H , and the rate of loss from compartment 5. The rate of loss of ^3H was higher than ^{14}C for the S-L group but in the NS groups the loss of ^3H was equal or less than ^{14}C . This could imply that different pathways of metabolism occurred.

For the NS-D group the flow rates between compartments 3 and 4 differed markedly to the other groups.

5.2.1.2 Differences between groups:

In Table 17 flow rates are expressed as fractions of those of the S-L group.

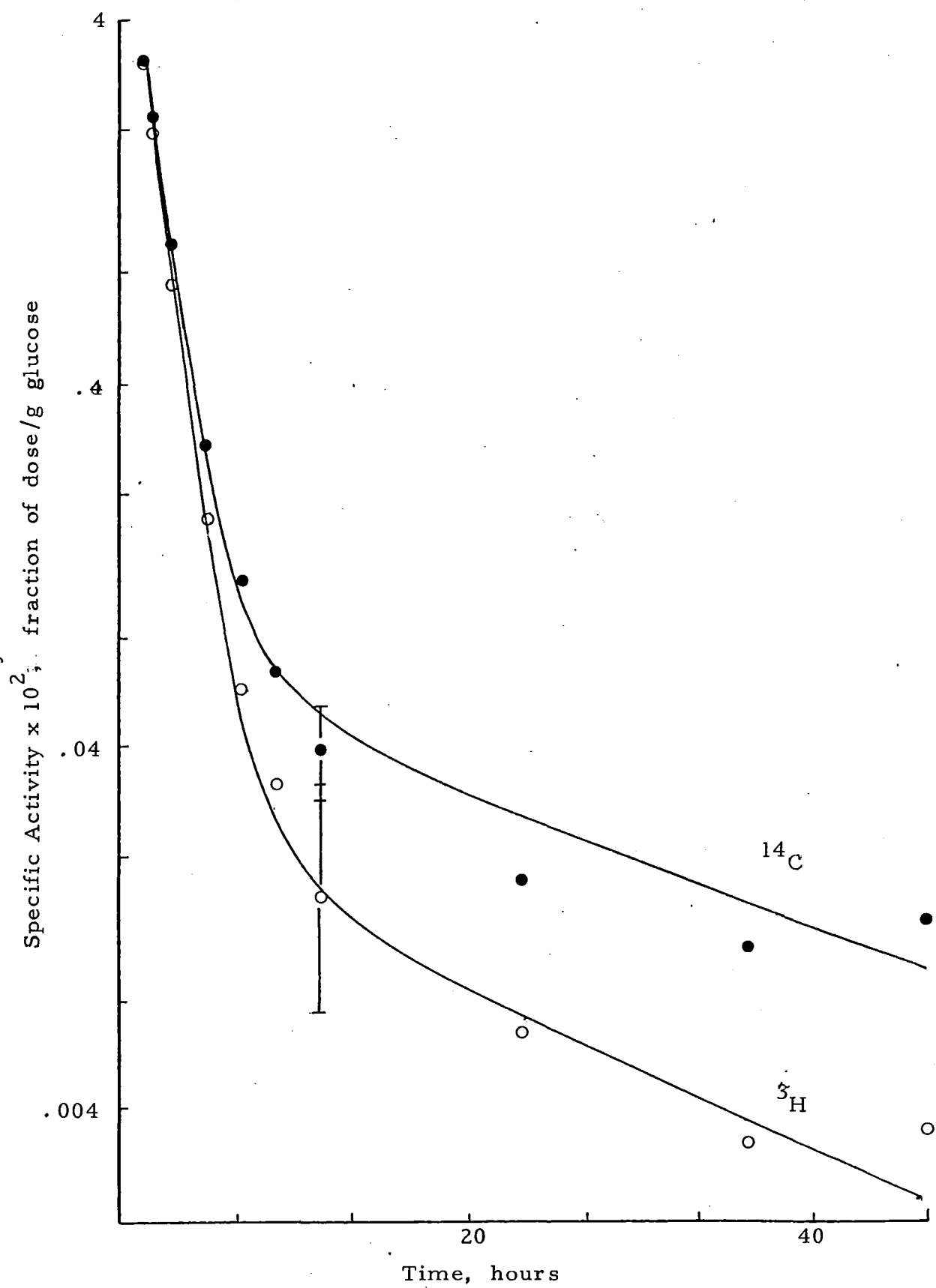
The NS-L differed in that L(4, 3) and L(3, 4) of ^{14}C were higher than the S-L group. This implies that there is a faster turnover of

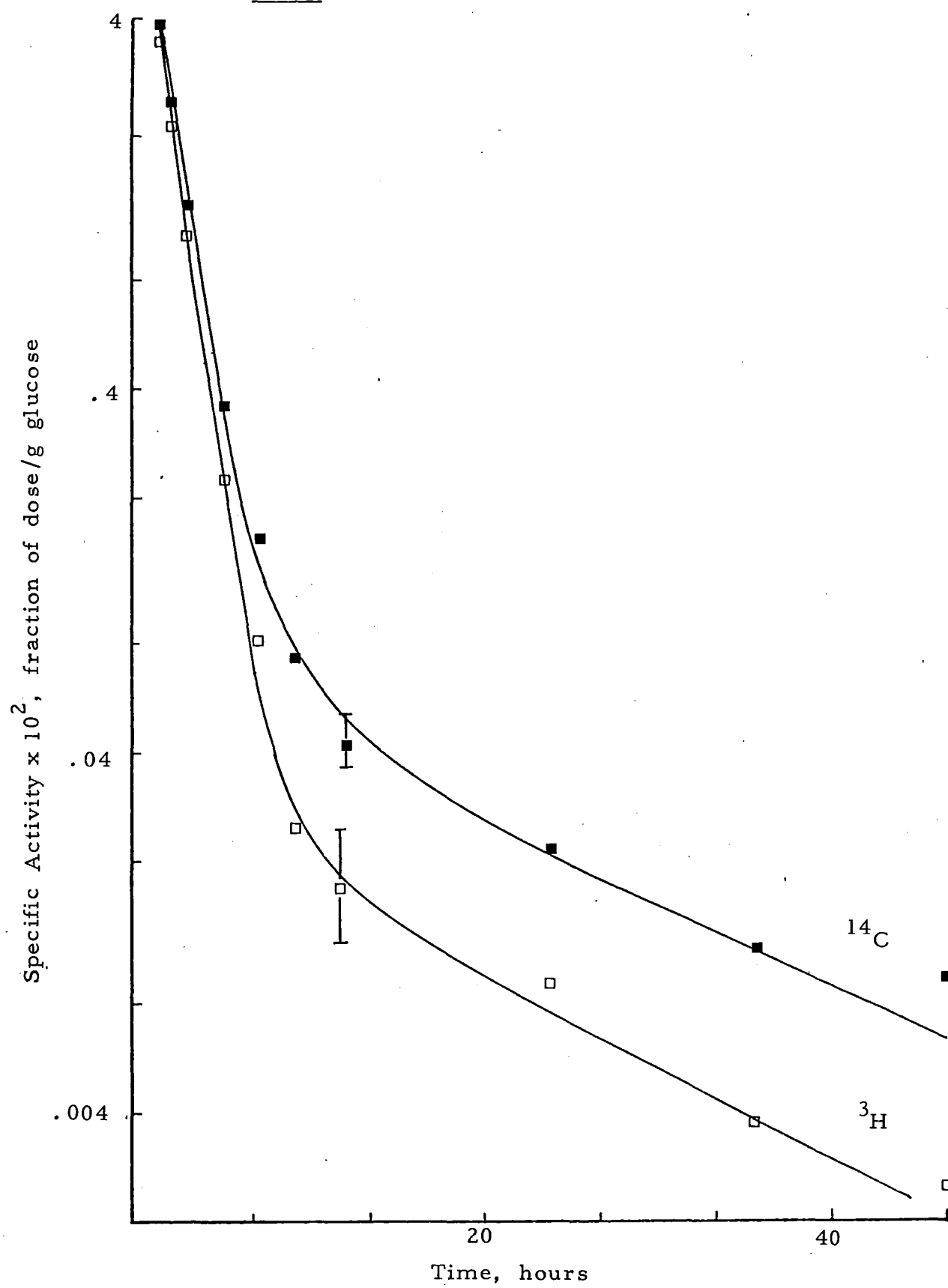
Fig. 18: Plasma glucose specific activity in fed ewes, susceptible or non-susceptible to pregnancy toxaemia, following the injection of $[U-^{14}C]$, $[6-^3H]$ glucose.

The ewes were classified on their response to a subsequent period of starvation:

- a) Susceptible with live lambs, S-L (N = 6)
- b) Non-susceptible with live lambs, NS-L (N = 6)
- c) Non-susceptible with dead lambs, NS-D (N = 3)

The points are the geometric means and the fitted curves are the sum of five exponentials. A 95% confidence interval is shown around the data.

a) S-L

b) NS-L

c) NS-D

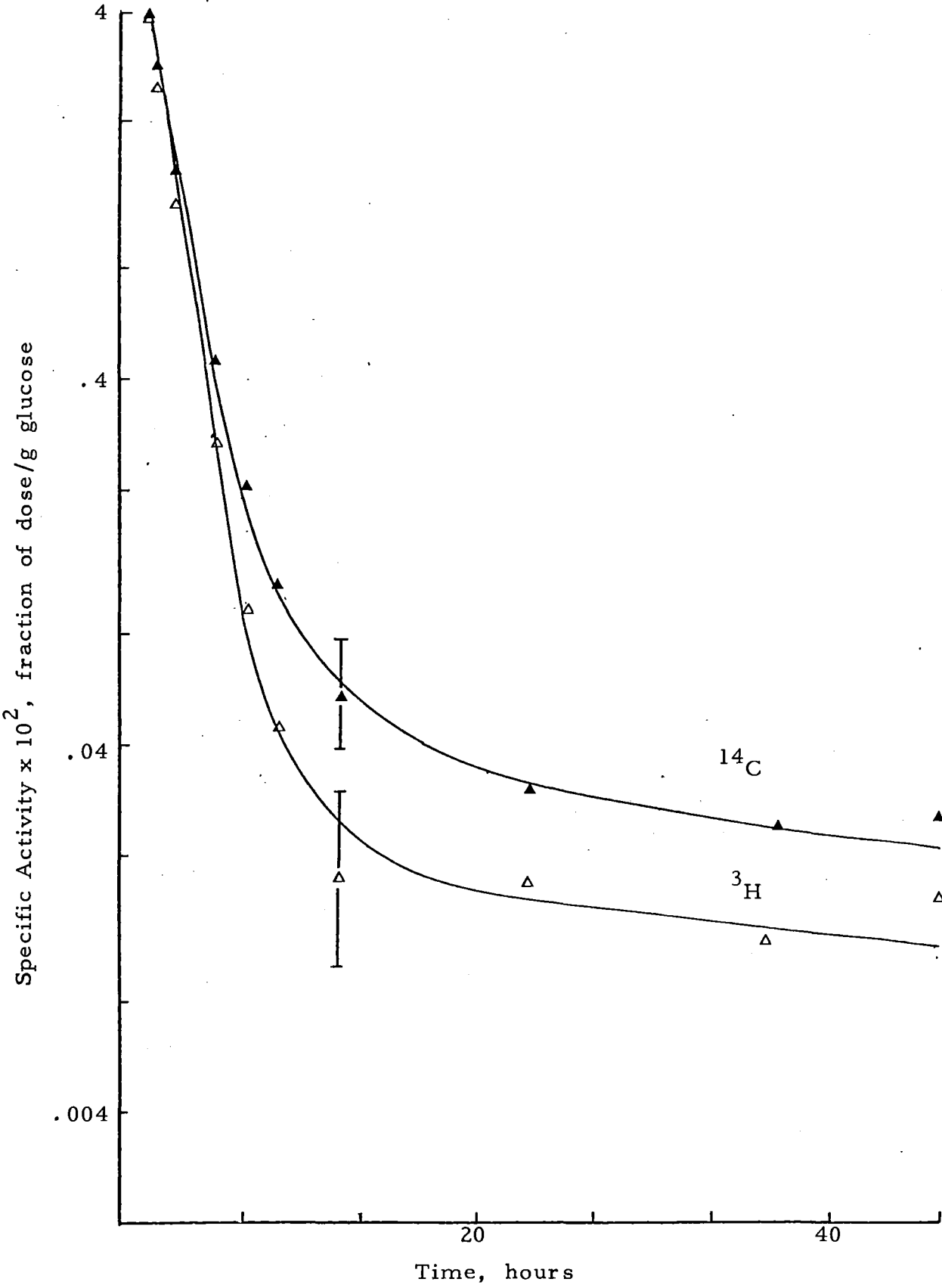


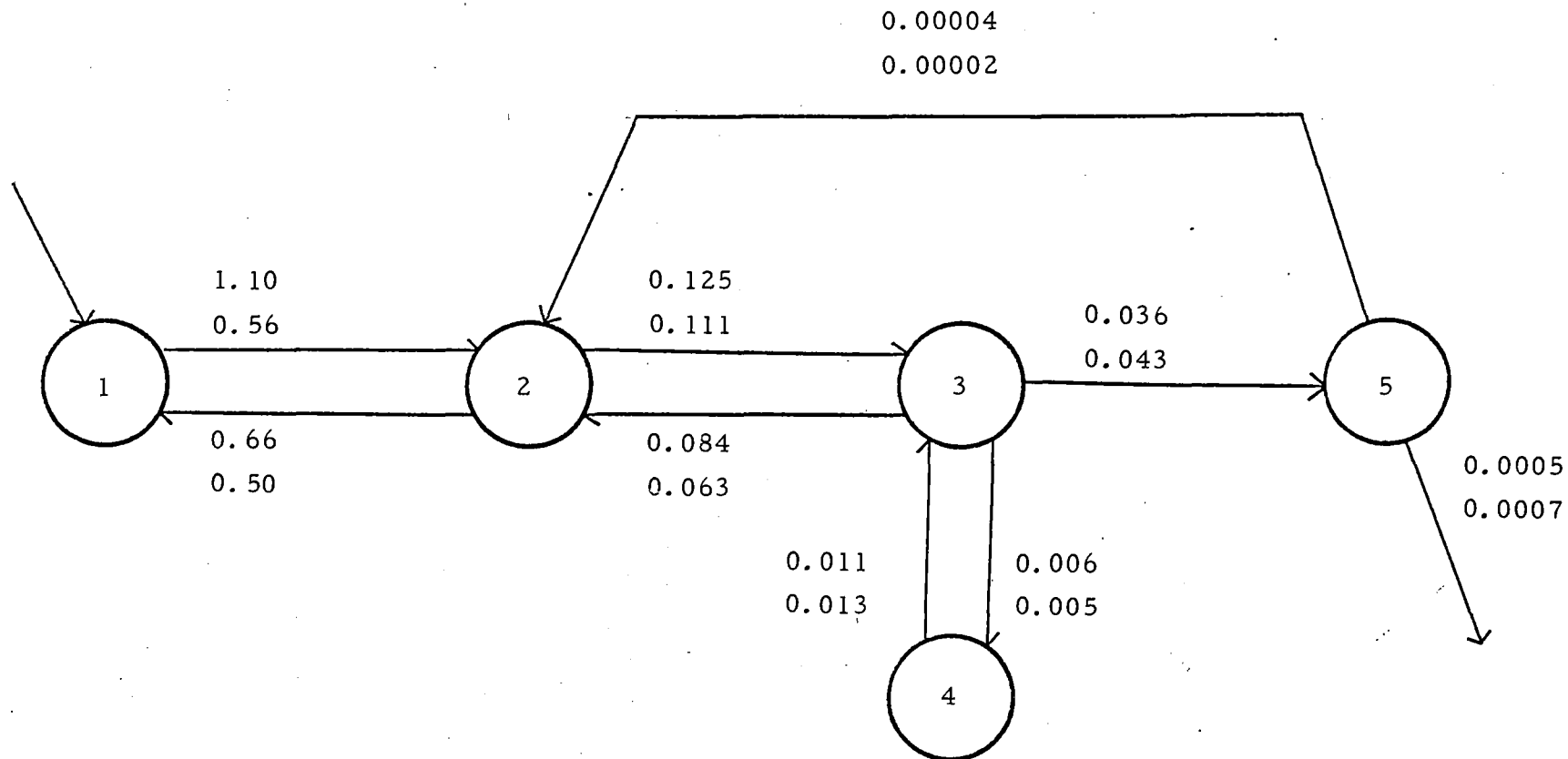
TABLE 15: Parameters of Fitted Curves: Each Group

Group (N)	Parameter	^{14}C	^3H
S-L (6)	Intercepts:		
	S(1, 2)	0.36016E-03	0.21421E-03
	S(1, 3)	0.48928E-04	0.13263E-03
	S(1, 4)	0.99128E-04	0.89011E-04
	S(1, 5)	0.94913E-04	0.27864E-04
	S(1, 6)	0.82744E-06	0.24666E-06
	Slopes:		
	L(0, 2)	0.17429E+01	0.11712E+01
	L(0, 3)	0.25699E-01	0.20926E-01
	L(0, 4)	0.15593E+00	0.16137E+00
	L(0, 5)	0.13654E-01	0.10052E-01
	L(0, 6)	0.76098E-03	0.84210E-03
NS-L (6)	Intercepts:		
	S(1, 2)	0.28092E-03	0.11614E-03
	S(1, 3)	0.10734E-03	0.41177E-04
	S(1, 4)	0.86378E-04	0.65596E-04
	S(1, 5)	0.97303E-04	0.77018E-04
	S(1, 6)	0.78889E-06	0.33763E-06
	Slopes:		
	L(0, 2)	0.22307E+01	0.23195E+00
	L(0, 3)	0.22989E+00	0.46360E-01
	L(0, 4)	0.10946E-01	0.23168E-01
	L(0, 5)	0.37776E-01	0.12131E-01
	L(0, 6)	0.88912E-03	0.10339E-02
NS-D (3)	Intercepts:		
	S(1, 2)	0.11364E-03	0.10870E-03
	S(1, 3)	0.93589E-04	0.10535E-03
	S(1, 4)	0.98786E-04	0.85070E-04
	S(1, 5)	0.40321E-05	0.27824E-05
	S(1, 6)	0.36512E-06	0.22464E-06
	Slopes:		
	L(0, 2)	0.84686E+00	0.75574E+00
	L(0, 3)	0.10039E+00	0.12255E-01
	L(0, 4)	0.11143E-01	0.10187E+00
	L(0, 5)	0.38255E-02	0.69066E-02
	L(0, 6)	0.18455E-03	0.25313E-03

The ewes were classed, after a period of starvation, as susceptible (S) or non-susceptible (NS) to pregnancy toxæmia with live (L) or dead (D) lambs in utero at slaughter. The curves represent the sum of five exponentials.

Fig. 19: Fractional Flow Rates of Tracers Fitted Independently - Model B.

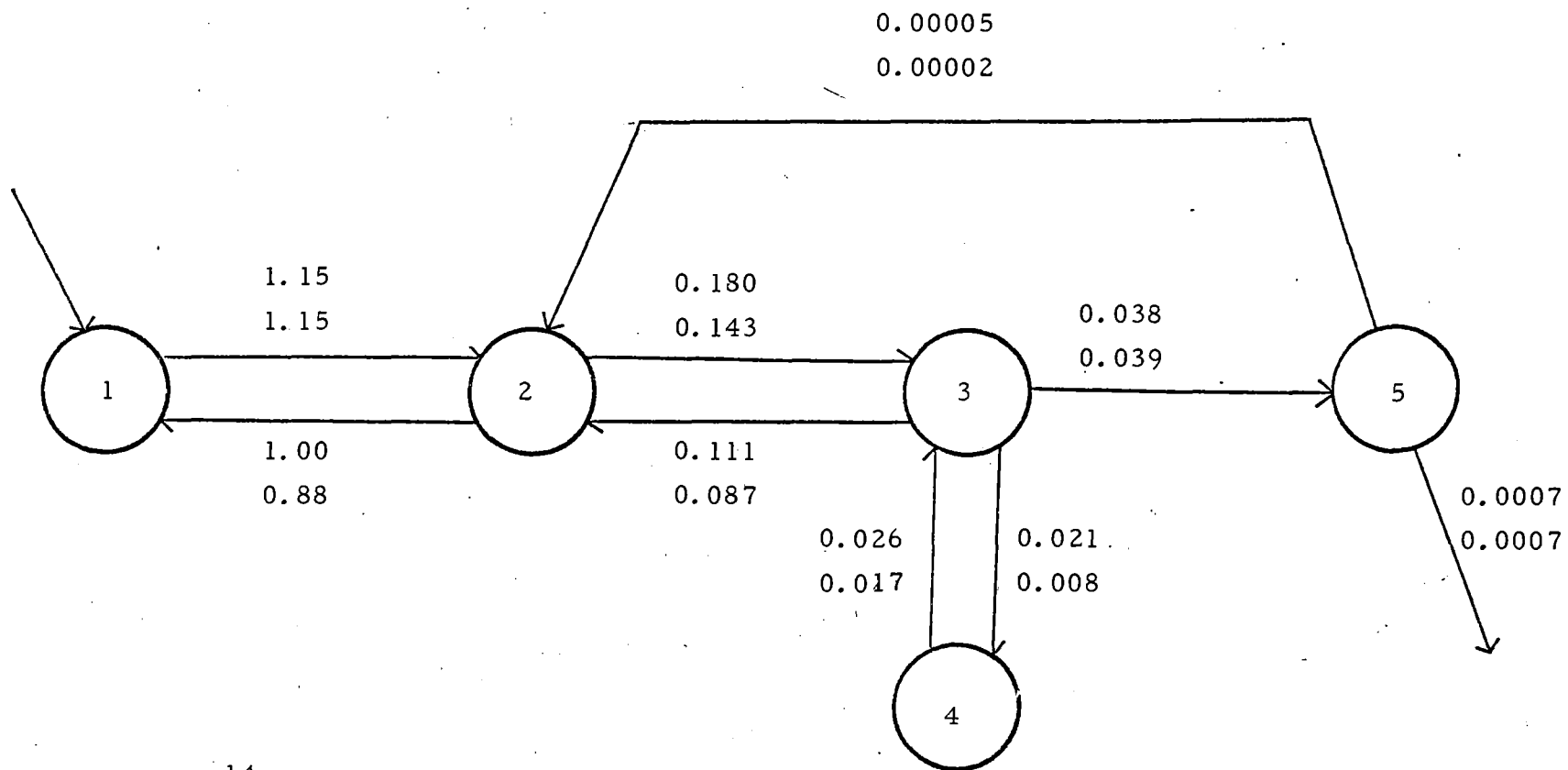
a) Susceptible Live



^{14}C
 ^3H

^{14}C K (1) 0.00061
 ^3H K (1) 0.00046

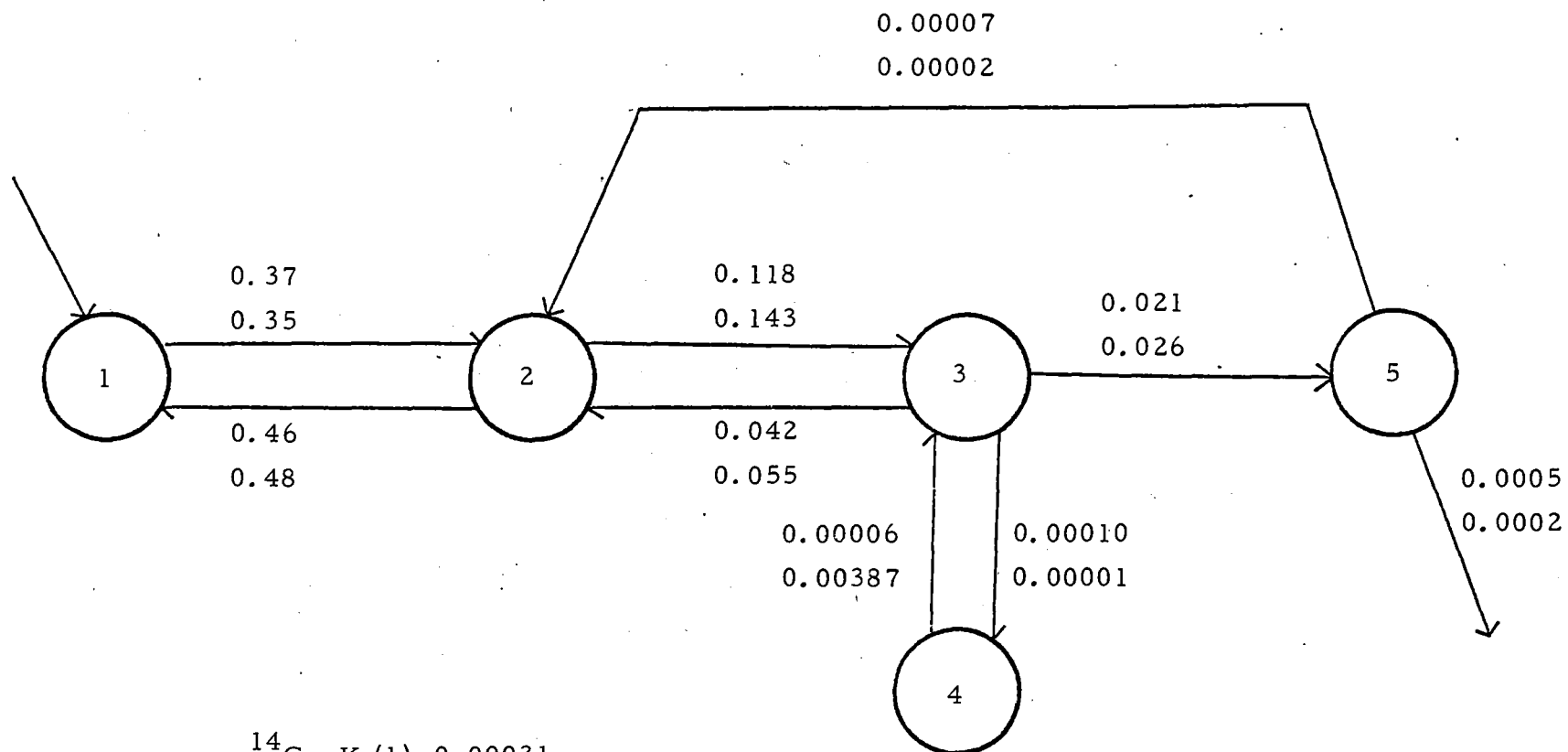
b) Non-Susceptible Live



^{14}C K (1) 0.00058

^3H K (1) 0.00060

c) Non-Susceptible Dead



^{14}C K (1) 0.00031

^3H K (1) 0.00031

TABLE 16: Tracer Differences Within Groups
(as Ratio $^{14}\text{C}/^3\text{H}$)

Parameter	Susceptible Live	Non-Susceptible	
		Live	Dead
L(2, 1)	1.98	1.00	1.04
L(1, 2)	1.32	1.14	0.96
L(3, 2)	1.13	1.26	0.83
L(2, 3)	1.33	1.27	0.76
L(4, 3)	1.19	2.51	9.92
L(3, 4)	0.84	1.53	0.02
L(5, 3)	0.85	0.97	0.79
L(2, 5)	2.04	2.88	2.79
L(0, 5)	0.69	1.05	2.30

The 5-compartment model describes glucose kinetics in pregnant ewes. The ratios of the parameters were determined after ^{14}C and ^3H data for each group of ewes were fitted to the model separately. The ewes were classed after a period of starvation as susceptible or non-susceptible to pregnancy toxaemia with live or dead lambs in utero at slaughter.

TABLE 17: Comparison of Fitted Parameters Between Groups
(as fraction of Susceptible-Live Group)

Parameter	Non-Susceptible			
	Live		Dead	
	^{14}C	^3H	^{14}C	^3H
L(2, 1)	1.04	2.06	0.33	0.63
L(1, 2)	1.52	1.76	0.70	0.96
L(3, 2)	1.44	1.29	0.95	1.29
L(2, 3)	1.32	1.39	0.50	0.88
L(4, 3)	3.24	1.54	0.02	0.002
L(3, 4)	2.45	1.35	0.01	0.30
L(5, 3)	1.05	0.92	0.57	0.61
L(2, 5)	1.42	1.01	1.82	1.33
L(0, 5)	1.52	1.00	1.04	0.31

The 5-compartment model describes glucose kinetics in pregnant ewes. The parameter values were determined by fitting ^{14}C and ^3H data for each group of ewes to the model.

The ewes were classed, after a period of starvation, as susceptible or non-susceptible to pregnancy toxæmia with live or dead lambs in utero at slaughter.

carbon compounds in compartment 4 in the NS-L group. In the NS-D group these two flow rates were much lower, but for both tracers, which could imply the label was still on glucose.

The differences observed in $L(2, 1)$ could be the result of early sampling being complicated by circulation of tracer.

5.2.3 FITTING THE TRACER DATA SIMULTANEOUSLY:

In order to examine the differences between tracers and determine which flows contributed most to the differences observed, the data of all sheep were fitted to model B and selected flow rates were allowed to vary independently for the two tracers, while the others were constrained to be the same.

Consistent differences occurred between the observed and calculated values, even allowing $L(0, 5)$, $L(2, 5)$ and $L(3, 4)$ to vary independently. It was concluded that modifications were required to the model before both the tracers would fit simultaneously. These modifications are discussed in the Discussion section.

5.3 SUMMARY:

A 5-compartment model was proposed to explain glucose kinetics in twin-pregnant ewes. Three compartments represented ECF and the other two, products and precursors of glucose metabolism. The model incorporates a recycling flow, and 8% of the carbon and 3% of the tritium, lost from the system was recycled.

The model was sufficient to explain tracer movements separately but was inadequate when the tracers were forced to fit simultaneously.

Data from S and NS groups of ewes were fitted separately and the results compared. The main differences between tracers were in the amount of label recycled and the amount lost from the system, and

the main difference between groups was movement of tracer between compartments 3 and 4.

6. GLUCOSE TURNOVER OF STARVED SHEEP

Primed continuous infusions were performed on the ewes once they were shown to be S or NS to OPT. This method was chosen for its relative simplicity in time to perform and number of samples for analysis.

The priming was considered necessary, to reach a plateau specific activity more rapidly, as many of the sheep were recumbent when the experiment was performed.

Two tracers, [^{14}C] and [^3H] glucose, were used such that an estimate of the recycling of ^{14}C label could be obtained.

6.1 NON-PREGNANT EWES:

The results of experiments undertaken in non-pregnant ewes during 10 days of starvation are shown in Table 18. Turnover rate decreased on starvation and the reduction was significant when the results of ewes starved more than 4 days were combined. After 4 days, turnover decreased by 26%, from 2.2 to 1.62 g/kg/d, and after 10 days the reduction was 30%, at 1.55 g/kg/d. There was no increase in recycling, measured by the difference in turnover calculated by the ^{14}C and ^3H tracers.

6.2 PREGNANT EWES SUSCEPTIBLE AND NON-SUSCEPTIBLE TO OPT:

Plasma glucose of ewes with live lambs was lower than those with dead lambs (Table 19) and also lower than the non-pregnant ewes starved more than 4 days. Ewes with dead lambs, regardless of whether or not they were showing symptoms of OPT, tended to be hyperglycaemic.

TABLE 18: Glucose Turnover of Non-Pregnant Ewes

Days Starve	No.	LW (kg)	Plasma Glucose (mg/dl)		Glucose Turnover (g/kg/d)	
			Day 0	Slaughter	¹⁴ C	³ H
1- 2	3	52 ± 1	59 ± 12	65 ± 21	2.20 ± 0.52 ^a	2.20 ± 0.53 ^a
4- 5	3	48 ± 1	61 ± 6	76 ± 11	1.62 ± 0.07 ^{ab}	1.58 ± 0.02 ^{ab}
9-10	3	53 ± 7	72 ± 11	72 ± 13	1.55 ± 0.14 ^{ab}	1.52 ± 0.17 ^{ab}
> 4	6	50 ± 5	67 ± 10	74 ± 11	1.59 ± 0.10 ^b	1.55 ± 0.11 ^b

Mean ± S.D.

There was no significant difference between the means based on the t-test (t.05).

When the two groups of ewes starved >4 days were pooled the glucose turnover measured by ¹⁴C and ³H were significantly (t.05) lower than ewes starved 1-2 days.

Glucose turnover was measured by the primed continuous infusion of [U-¹⁴C]- and [6-³H] glucose, and the ewes were starved for periods up to 10 days.

TABLE 19: Glucose Turnover of Starved Pregnant Ewes

Group	No.	LW (kg)	Plasma Glucose (mg/dl)	Glucose Turnover (g/kg/d)		Recycling (%)
				^{14}C	^3H	
S-L	4	39 \pm 4	42 \pm 8 ^a	2.67 \pm 0.53 ^a	2.79 \pm 0.58 ^a	4.1 \pm 2.1
S-D	5	40 \pm 4	116 \pm 91 ^{ab}	6.90 \pm 3.42 ^b	7.49 \pm 3.47 ^b	7.8 \pm 7.3
NS-L	1	47	46	4.28	4.44	3.6
NS-D	6	44 \pm 8	119 \pm 75 ^b	6.42 \pm 2.96 ^b	6.73 \pm 3.27 ^b	3.6 \pm 5.0

Mean \pm S.D.

Means with different superscripts differ at 5% level, based on Wilcoxon's two-sample rank test.

Glucose turnover was measured by the primed continuous infusion of [U- ^{14}C] and [6- ^3H] glucose. Ewes were susceptible (S) or non-susceptible (NS) to toxæmia with live (L) or dead (D) lambs in utero at slaughter.

Glucose turnover of the S-L group was half (2.67 g/kg/d) that of the NS-L group (4.28 g/kg/d), and was similar to the turnover recorded in non-pregnant ewes starved 1-2 days (2.20 g/kg/d).

Ewes with dead lambs, S-D and NS-D both had significantly higher turnover rates than S-L. Thus plasma glucose and glucose turnover appeared to increase on death of the lamb.

Recycling between the groups was similar, although a large variability was recorded.

6.3 SUMMARY:

A summary of the turnover rates, and % recycling is shown in Table 20.

7. HEPATOCYTE GLUCOSE PRODUCTION OF STARVED SHEEP

7.1 NON-PREGNANT EWES:

Glucose production rates of hepatocytes, from non-pregnant ewes starved for 4-10 days, are shown in Table 21. There was no difference between the production rates of ewes starved for 4-5 days and ewes starved for 9-10 days, and so the results were pooled to provide estimates for starved non-pregnant ewes.

The substrates are ranked in Table 22. Glucagon tended to increase the production rates, but as the basal rate was also higher with glucagon, it seems that the cells responded to hormonal stimulation by gluconeogenesis and glycogenolysis.

TABLE 20: Summary: Glucose Turnover of Starved Ewes

Ewes	Starvation Period (Days)	No.	Plasma Glucose (mg/dl)	Glucose Turnover (g/kg/d)		Recycling (%)
				^{14}C	^3H	
Pregnant ^a						
S-L	5	4	42	2.67	2.79	4.1
S-D	4	5	116	6.90	7.49	7.8
NS-L	10	1	46	4.28	4.44	3.6
NS-D	10	6	119	6.42	6.73	3.6
Non-Pregnant	1-2	3	65	2.20	2.20	0.0
Foetuses ^b	>4	6	74	1.59	1.55	
0 Dead	4-5	5	27	2.39	2.55	6.5
1 Dead	4-5	4	42	3.96	4.27	7.0
2 Dead	4-5	3	99	4.82	5.65	11.9

Glucose turnover was measured by the primed continuous infusion of [U- ^{14}C] and [6- ^3H] glucose.

^a Ewes were susceptible (S) or non-susceptible (NS) to pregnancy toxæmia with live (L) or dead (D) lambs in utero at slaughter.

^b Twin-pregnant ewes were starved for four days, and then 0, 1 or 2 foetuses were killed in utero by tightening nooses which had been surgically placed around each umbilical cord.

TABLE 21: Hepatocyte Glucose Production - Non-Pregnant Ewes.

Starvation Period (Days)	No.	Glucose Production ($\mu\text{mol/g/min}$)								
		Substrate								
		B	gn + B	P	gn + P	L	gn + L	A	Gc	gn + Gc
4- 5	3	1.30 \pm 1.94	1.29 \pm 1.22	2.21 \pm 3.18	1.73 \pm 1.77	0.86 \pm 0.81	1.55 \pm 0.39	1.80 \pm 2.30	1.03 \pm 0.98	1.71 \pm 1.68
9-10	3	0.56 \pm 1.09	2.73 \pm 1.82	1.86 \pm 2.10	2.53 \pm 1.71	1.70 \pm 2.91	1.78 \pm 2.18	0.84 \pm 0.40	2.58 \pm 2.29	2.01 \pm 1.24
Pooling >4	6	0.94 \pm 1.46	2.01 \pm 1.60	2.04 \pm 2.42	2.13 \pm 1.61	1.28 \pm 1.96	1.66 \pm 1.64	1.33 \pm 1.57	1.80 \pm 1.79	1.86 \pm 1.33

Mean \pm S.D.

There was no difference between the variance of the groups based on the F-test, so the results were pooled.

B - Basal, P - Propionate, L - Lactate, A - Alanine, Gc - Glycerol,
G - Glutamine, gn - glucagon.

Hepatocytes were isolated from the caudate lobe by perfusion with collagenase, and incubated for 30 min with substrate (10 mM) and glucagon (10^{-8} M)

TABLE 22: Hepatocyte Glucose Production of Non-Pregnant Ewes -
Ranking of Treatments.

Rank	Ewes Starved 4-10 Days		
Lowest			
1	Basal		
2	Lactate		
3	[Propionate	
4		Alanine	
5	Lactate	+	glucagon
6	Glycerol		
7	Glycerol	+	glucagon
8	Propionate	+	glucagon
9	Basal	+	glucagon
Highest			

There was no difference between substrates based on the non-parametric Chi-squared test.

Hepatocytes were isolated from the caudate lobe by perfusion with collagenase, and incubated for 30 min with substrate (10 mM) and glucagon (10^{-8} M)

7.2 PREGNANT EWES SUSCEPTIBLE AND NON-SUSCEPTIBLE TO OPT:

The hepatocyte glucose production rates are shown in Table 23, the glucagon stimulation is shown in Table 24, and the results for individual sheep are shown in Appendix 5, Table 37.

1977:

S-L ewes had low hepatocyte glucose production rates and the rates with basal, propionate, lactate and alanine were significantly lower than NS-L ewes.

All glucose production rates of the S-L group were significantly lower than the NS-D group. The NS-D ewes tended to have higher rates of production than NS-L, but also had larger variability and only the rates with propionate and alanine differed significantly between the groups.

Glucagon stimulation, as the increase over production with the substrate alone, is shown in Table 24. NS-L had significantly greater stimulation than S-L, with glucagon over basal and with alanine.

The stimulation of production was even greater in the NS-D group.

Thus the hepatocytes from S ewes do not appear to be responsive to glucagon, while the response of hepatocytes from NS-L is less than NS-D.

The effect of substrates is shown in Table 25a. Substrates did have an effect in the NS groups, and propionate and lactate in the presence of glucagon gave the highest production rates. Low glucose production rates were recorded with alanine in all groups.

1978:

The glucose production rates of individual ewes are shown in Table 37, Appendix 5, and are summarized in Table 23. A ewe

TABLE 23: Hepatocyte Glucose Production of Starved Pregnant Ewes.

1977

Group	No.	Glucose Production ($\mu\text{mol/g/min}$)				
		B	P	L	G	A
S-L	7	0.11 ± 0.18^a	0.15 ± 0.42^a	0.13 ± 0.41^a	0.04 ± 0.47^a	0.03 ± 0.27^a
NS-L	6	0.65 ± 0.33^b	1.22 ± 0.75^b	1.38 ± 0.68^b	0.77 ± 0.56^a	0.85 ± 0.70^b
NS-D	3	1.03 ± 0.28^b	4.84 ± 3.31^c	3.13 ± 2.62^b	5.30 ± 3.11^b	2.71 ± 0.88^c

Mean \pm S.D.

1978

Group	No.	B	P	L	Gc	A
S-L	3	0.20 ± 0.17^a	0.68 ± 0.74^a	0.67 ± 0.64^a	0.25 ± 0.16^a	0.34 ± 0.35^a
S-D	4	0.40 ± 0.53^a	0.61 ± 0.79^a	0.49 ± 0.64^a	0.32 ± 0.47^a	0.41 ± 0.50^{ab}
NS-L	1	0.02	0.08	0.07	0.04	0.12
NS-D	2	4.29 ± 3.29^b	5.12 ± 4.04^b	4.29 ± 2.82^b	3.57 ± 3.56^b	3.85 ± 4.30^b

Mean \pm S.D.

Means with different superscripts differ significantly at 5% level, based on Wilcoxon's two-sample rank test.

Ewes were susceptible (S) or non-susceptible (NS) to pregnancy toxæmia with live (L) or dead (D) lambs in utero at slaughter.

Hepatocytes were isolated from the caudate lobe by perfusion with collagenase, and incubated for 30 min with substrate (10 mM).

B - Basal P - Propionate L - Lactate G - Glutamine
A - Alanine Gc - Glycerol

TABLE 24: Stimulation of Hepatocyte Glucose Production by Glucagon

1977

Group	No.	Glucose Produced Over Substrate Alone ($\mu\text{mol/g/min}$)						
		10 ⁻⁷ M Glucagon		10 ⁻⁸ M Glucagon				
		+ B	+ P	+ B	+ P	+ L	+ G	+ A
S-L	7	-0.07 ^a ± 0.19	0.03 ^a ± 0.33	0.04 ^a ± 0.07	0.05 ± 0.26	-0.02 ± 0.06	0.01 ± 0.10	0.01 ^a ± 0.22
NS-L	6	0.74 ^b ± 0.52	0.27 ^a ± 0.32	0.74 ^b ± 0.65	0.34 ± 0.42	0.17 ± 0.25	0.48 ± 0.53	0.32 ^b ± 0.33
NS-D	3	4.01 ^c ± 0.45	1.46 ^b ± 0.70	3.92 ^c ± 1.52	1.88 ± 3.45	1.50 ± 3.98	1.23 ± 1.99	2.50 ^{ab} ± 2.94

1978

Group	No.	10 ⁻⁸ M Glucagon				
		+ B	+ P	+ L	+ Gc	+ A
S-L	3	-0.03 ^a ± 0.07	-0.19 ^a ± 0.17	-0.20 ^a ± 0.12	-0.09 ± 0.15	-0.01 ^a ± 0.07
S-D	4	0.01 ^{ab} ± 0.14	0.05 ^a ± 0.10	-0.05 ^{ab} ± 0.18	-0.14 ± 0.15	0.07 ^a ± 0.23
NS-L	1	0.10	0.08	0.04	-0.06	-0.05
NS-D	2	0.81 ^b ± 1.07	1.00 ^b ± 0.64	0.90 ^b ± 1.24	0.63 ± 1.63	1.28 ^b ± 0.26

Mean ± S.D.

Means with different superscripts differ at 5% level, based on Wilcoxon's two-sample rank test.

Ewes were susceptible (S) or non-susceptible (NS) to pregnancy toxemia with live (L) or dead (D) lambs in utero at slaughter.

Hepatocytes were isolated from the caudate lobe by perfusion with collagenase, and incubated for 30 min with substrate (10 mM) and glucagon.

B - Basal P - Propionate L - Lactate G - Glutamine
A - Alanine Gc - Glycerol

TABLE 25: Hepatocyte Glucose Production of Pregnant Ewes -
Ranking of Treatments.

Ewes were susceptible or non-susceptible to pregnancy toxaemia with live or dead lambs in utero at slaughter.

Hepatocytes were isolated from the caudate lobes by perfusion with collagenase, and incubated for 30 min with substrate (10 mM) and glucagon (10^{-7} or 10^{-8} M).

A - Alanine,	B - Basal,	L - Lactate
P - Propionate,	Gc - Glycerol,	gn - glucagon

a) 1977

Rank	Susceptible Live (No.) (7)	Non-Susceptible	
		Live (6)	Dead (3)
Lowest			
1	A + 10^{-8} gn	B	B
2	B + 10^{-7} gn	A	[L
3	A	P	[A
4	L	A + 10^{-8} gn	[A + 10^{-8} gn
5	L + 10^{-8} gn	B + 10^{-8} gn	[P
6	B	B + 10^{-7} gn	[B + 10^{-7} gn
7	P + 10^{-8} gn	L	[B + 10^{-8} gn
8	P	P + 10^{-8} gn	L + 10^{-8} gn
9	P + 10^{-7} gn	P + 10^{-7} gn	P + 10^{-7} gn
10	B + 10^{-8} gn	L + 10^{-8} gn	P + 10^{-8} gn
Highest			

There was no difference between substrates in the susceptible-live group, based on non-parametric Chi-squared test.

In the non-susceptible groups, treatments did have an effect on glucose production rate. In the non-susceptible-live group, all substrates higher than lactate were significantly different from basal, and in the non-susceptible-dead group, all substrates except lactate were significantly different from basal, based on the non-parametric Chi-squared test at 5% level.

TABLE 25 (Continued)

b) 1978

Rank (No.)	Susceptible		Non-Susceptible
	Live (3)	Dead (4)	Dead (2)
Lowest			
1	B	Gc + gn	Gc
2	B + gn	Gc	[Gc + gn
3	Gc + gn	B + gn	[A
4	L + gn	B	B
5	[A	A	L
6	[A + gn	L + gn	P
7	[P + gn	L	[B + gn
8	[Gc	A + gn	[A + gn
9	P	P + gn	L + gn
10	L	P	P + gn
Highest			

There was no significant difference between substrates of the susceptible groups when tested separately, or pooled, based on the non-parametric Chi-squared test, nor between those of the non-susceptible group.

(132) in the NS group had production rates that were so different from the others in this group that the data from this liver have been excluded from the calculations.

No difference was found in hepatocyte glucose production rates by the S-L or S-D groups, and the rates are comparable with the S-L group of 1977.

Only one ewe was recorded in the NS-L group and, due to the variability in this type of experiment, no conclusions could be drawn. However the NS-D group had significantly higher production rates than the S groups with all substrates, and the rates are similar to those recorded in NS-D group of 1977.

The stimulation of production rates by glucagon is shown in Table 24. Only glucagon at 10^{-8} M was used, as this tended to give higher rates than 10^{-7} M. No stimulation was recorded in the S groups. The rates recorded in the NS-D group were significantly higher than the S groups with all substrates except glycerol, and were slightly less than those recorded in the previous year.

The ranking of substrates is shown in Table 25b. In contrast to 1977, substrates could not be shown to have had an effect. Production with alanine was again low and glycerol also had low rates of production in all groups. Lactate and propionate continued to give the highest rates and the marked effect of glucagon can be seen in the NS-D group.

7.3 SUMMARY:

A summary of glucose production rates for all sheep is shown in Table 26. The rates by hepatocytes from ewes with OPT, were significantly lower than those from ewes not showing signs of the disease, and the S ewes did not respond to the presence of 10^{-8} M glucagon. These results were obtained whether the lambs were dead or alive.

TABLE 26: Summary: Hepatocyte Glucose Production.

Group	No.	Glucose Produced ($\mu\text{mol/g/min}$)					
		B	$10^{-8}\text{gn} + \text{B}$	P	$10^{-8}\text{gn} + \text{P}$	L	A
1977:							
S-L	7	0.11	0.13	0.15	0.20	0.13	0.03
NS-L	6	0.65	1.39	1.22	1.56	1.38	0.85
NS-D	3	1.03	4.95	4.84	6.72	3.13	2.71
1978:							
S-L	3	0.20	0.18	0.68	0.49	0.67	0.34
S-D	4	0.40	0.41	0.61	0.65	0.49	0.41
NS-L	1	0.02	0.12	0.08	0.16	0.07	0.12
NS-D	2	4.29	5.10	5.12	6.12	4.29	3.85
Non-Pregnant > 4 days starve	6	0.94	2.01	2.04	2.13	1.28	1.33

Hepatocyte glucose production of ewes susceptible (S) or non-susceptible (NS) to pregnancy toxæmia with live (L) or dead (D) lambs in utero, and starved non-pregnant ewes.

Hepatocytes were isolated from the caudate lobe by perfusion with collagenase, and incubated for 30 min with substrate (10 mM) and glucagon (10^{-8} M).

B - Basal P - Propionate L - Lactate
A - Alanine gn - glucagon

Ewes not susceptible to the disease, with live lambs, had rates which were higher, and similar to starved non-pregnant ewes, while the highest production rates were recorded in NS ewes with dead lambs. The hepatocytes from these sheep were responsive to the presence of 10^{-8} M glucagon. Thus death of the lambs in the NS group appeared to raise the gluconeogenic potential of the hepatocytes.

8. GLUCOSE TURNOVER AND HEPATOCYTE GLUCOSE PRODUCTION.

In Figs 20 and 21 the in vivo glucose turnover rate, measured with [U- 14 C] glucose, has been drawn against the glucose production rate of the liver measured in vitro by incubating hepatocytes with glucose precursors.

Hepatocyte glucose production (μ mol glucose/g cells dry weight/min) was converted to g glucose/day per animal by estimating the dry weight of cells in each liver. The liver dry weight, calculated from liver weight and % dry matter determinations, was multiplied by a factor of 0.85, as hepatocytes constitute about 85% of the liver by volume (see Krebs et al., 1974).

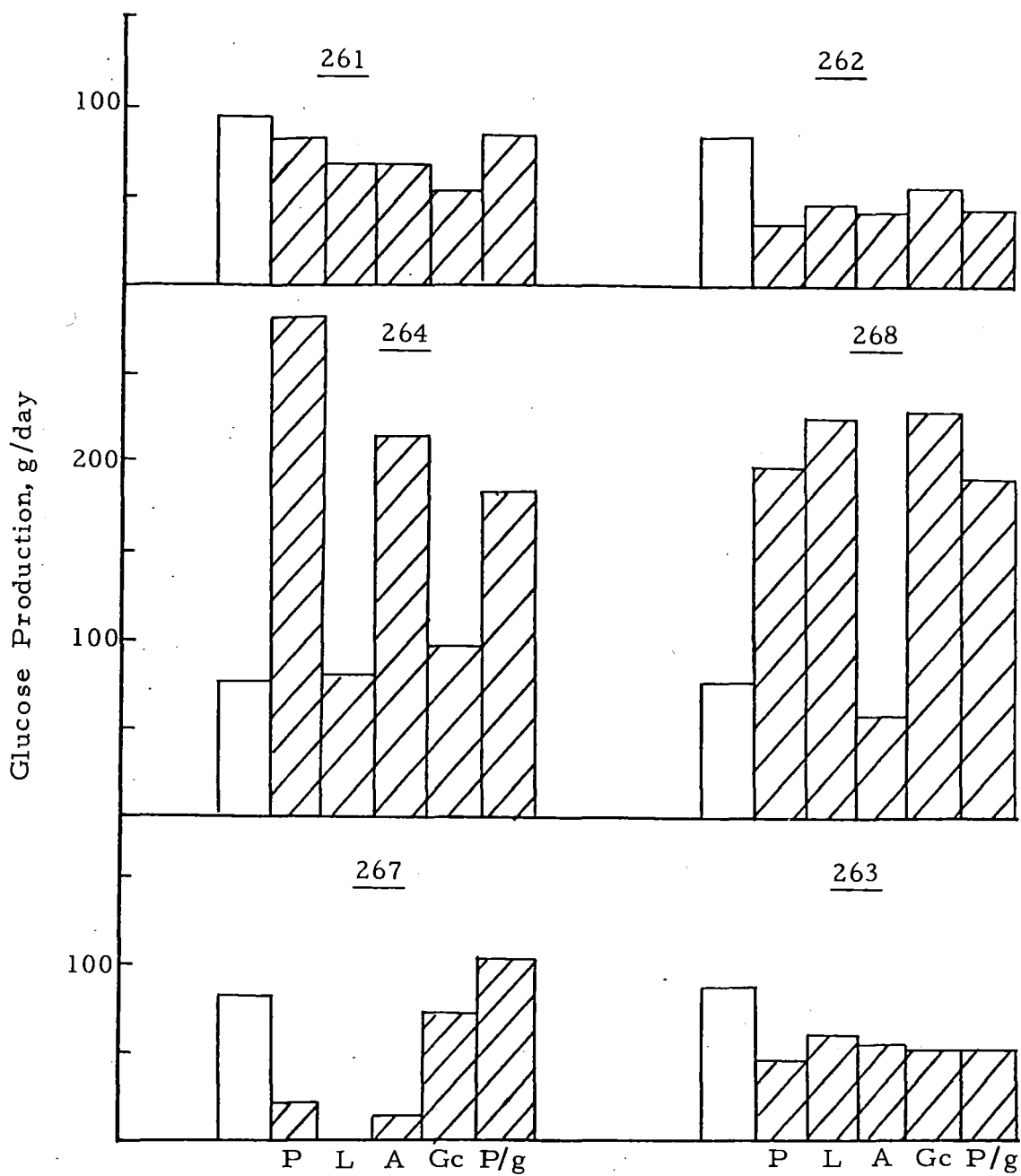
The values for the individual starved ewes are shown in Fig. 20, and the group means in Fig. 21.

9. EFFECT OF KILLING THE FOETUSES IN UTERO.

Foetuses have been killed in utero in previous studies by occluding the umbilical cord. Dawes et al. (1959) in sheep and Randall (1979) in pigs, monitored the changes in foetal metabolism after the occlusion. This study monitored the effect of killing the foetuses on maternal metabolism.

a)

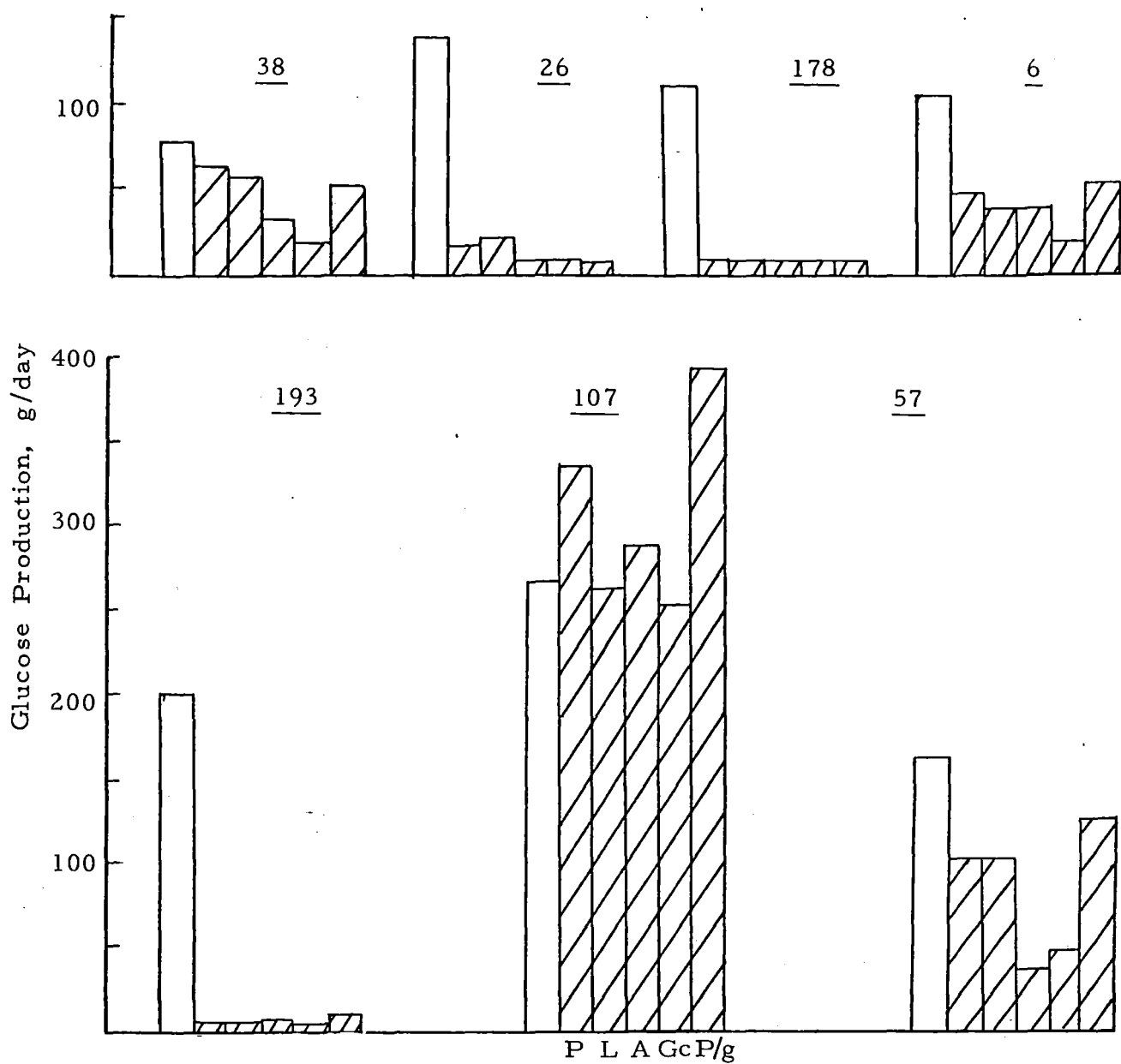
175.



□ Glucose turnover by $[U-^{14}C]$ glucose infusion

▨ Hepatocyte glucose production

b)



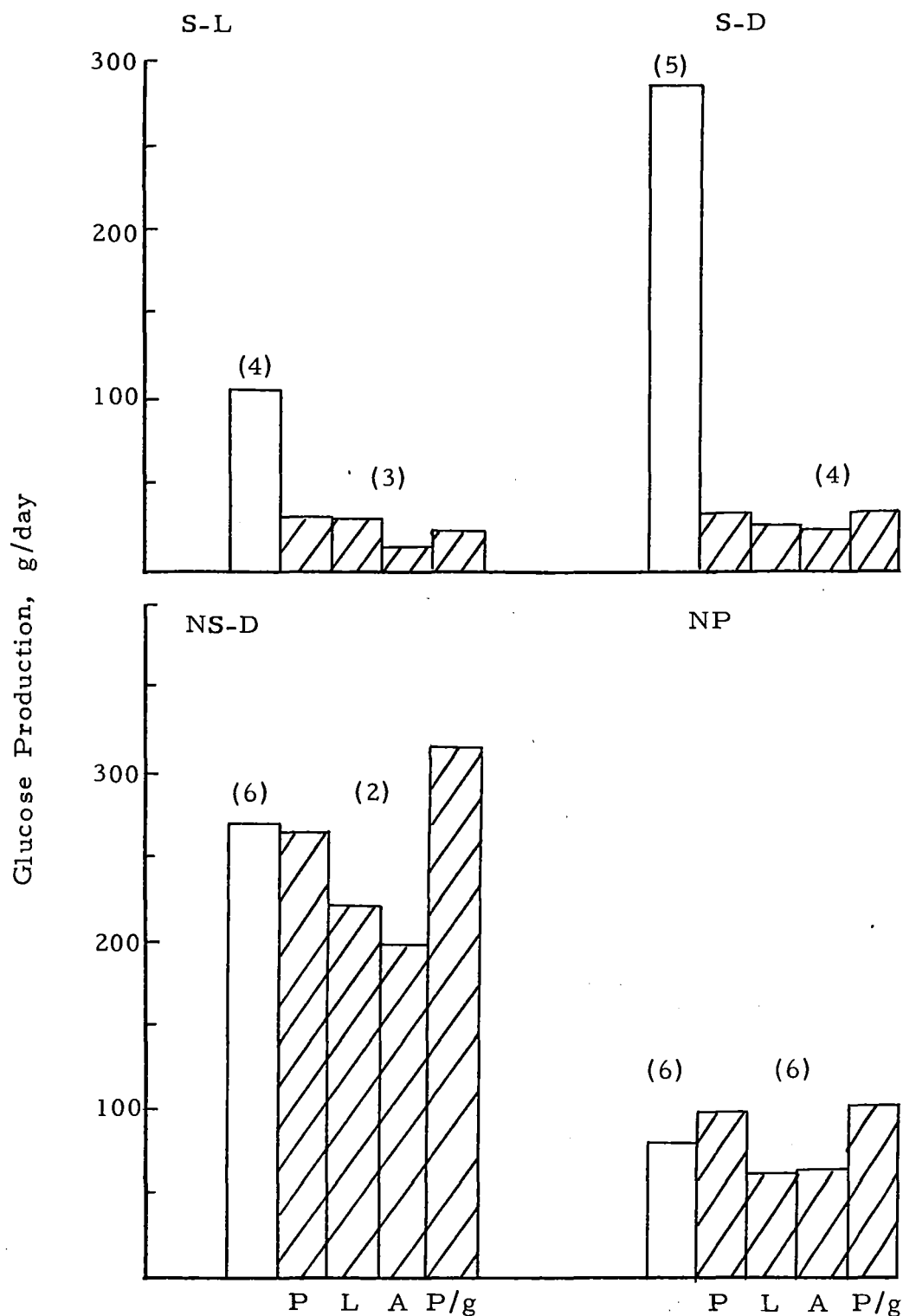


Fig. 21: Glucose production determined by primed continuous infusion of [¹⁴C] glucose and by incubation of hepatocytes . The ewes were susceptible (S) or non-susceptible (NS) to pregnancy toxæmia, with dead (D) or live (L) lambs, or non-pregnant (NP) and starved 4-10 days.

(N) - number of ewes.

Substrates: (10 mM), P - Propionate, L - Lactate, A - Alanine, P/g - Propionate with 10⁻⁸ M glucagon.

The results are classified according to the state of the lambs as 0 dead, one dead or two dead.

9.1 PLASMA AND LIVER METABOLITES:

The effect on plasma glucose over 24 hours after foetal death is shown in Fig. 22. Glucose concentrations were significantly lower in ewes with both lambs alive (Table 27). The figure for ewes with both lambs dead is higher, but the large SD shows that the response was variable between sheep.

The plasma acetoacetate concentration was highest in ewes with live lambs. The level (1.26 mM) was 1.5 times that of ewes with one lamb alive, and 3 times that of ewes with two dead lambs (0.43 mM). No difference could be shown between the groups on liver fat or glycogen although glycogen levels tended to be lower in ewes with live lambs.

Killing the foetuses in utero therefore significantly raised plasma glucose and tended to reduce plasma acetoacetate concentrations.

9.2 GLUCOSE TURNOVER:

Glucose turnover of ewes before and after foetal death, measured by primed continuous infusion of [^{14}C]- and [^3H] glucose, is shown in Table 28.

Killing one and two foetuses, progressively raised the glucose turnover. Turnover in ewes with two dead foetuses was double that of ewes with two live lambs. The differences were not significant, due to the large variability between sheep.

The individual values are shown in Table 29. Glucose turnover increased in 3 ewes out of 4 in which the foetuses were killed.

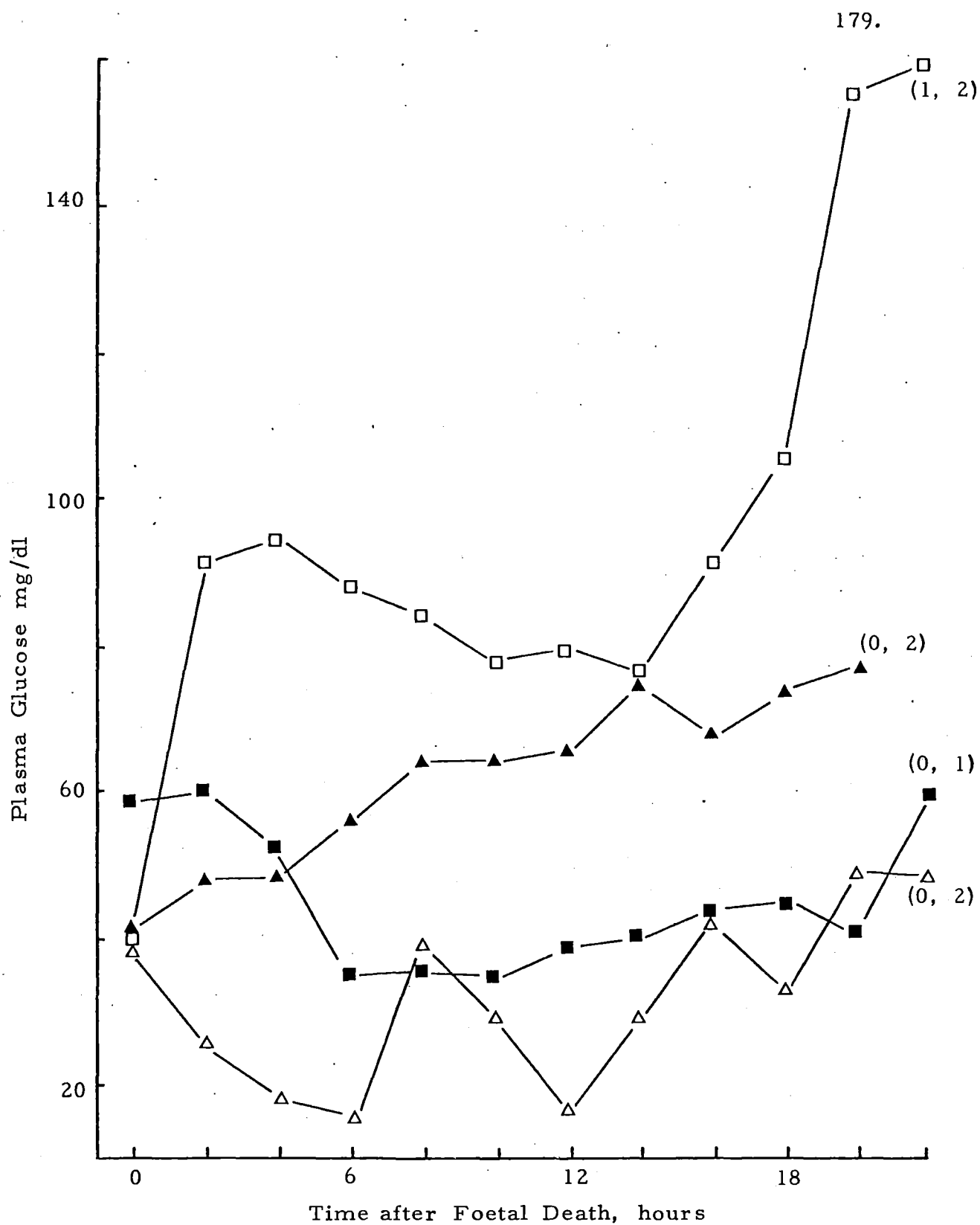


Fig. 22: Changes in plasma glucose in twin-pregnant ewes after killing foetuses in utero.

Sheep number: 14 (▲), 47 (△), 235 (□), 253 (■).

(N0, N1) - (number of dead foetuses at 0 hours, and at ewe slaughter)

TABLE 27: Effect of Killing Foetuses In Utero
on Metabolite Levels

Foetuses	No.	PLASMA		LIVER	
		Glucose (mg/dl)	AcAc (mM)	Fat (% wet wt)	Glycogen (mg/g)
0 Dead	5	27 ± 11 ^a	1.26 ± 0.72	13.5 ± 2.9	1.7 ± 1.6
1 Dead	4	42 ± 7 ^b	0.83 ± 0.23	20.3 ± 3.8	8.0 ± 5.2
2 Dead	3	99 ± 63 ^b	0.43 ± 0.33	13.4 ± 9.0	7.3 ± 4.8

Mean ± S.D.

Means with different superscripts differ significantly at 5% level, based on the t-test.

Twin-pregnant ewes were starved for four days and then 0, 1 or 2 foetuses were killed in utero by tightening nooses, which had been surgically placed around each umbilical cord. The ewes were slaughtered after 24 hours.

AcAc - Acetoacetate

TABLE 28: Effect of Killing Foetuses In Utero
on Glucose Turnover.

Foetuses	No.	Wt. (kg)	Glucose Turnover (g/kg/d)		Recycling (%)
			^{14}C	^3H	
0 Dead	5	47 ± 6	2.39 ± 1.17	2.55 ± 1.24	6.5 ± 1.2 ^a
1 Dead	4	45 ± 9	3.96 ± 1.56	4.27 ± 1.68	7.0 ± 2.7 ^{ab}
2 Dead	3	42 ± 10	4.82 ± 4.94	5.65 ± 6.00	11.9 ± 3.9 ^b

Mean ± S.D.

Means with different superscripts differed significantly at 5% level, based on the t-test.

Twin-pregnant ewes were starved for four days and then 0, 1 or 2 foetuses were killed in utero by tightening nooses which had been surgically placed around each umbilical cord.

Glucose turnover was measured by the primed continuous infusion of [U- ^{14}C] and [6- ^3H] glucose, before and 24 hours after foetal death.

TABLE 29: Effect of Killing Foetuses In Utero on Glucose Turnover of Individual Ewes.

Ewe	Glucose Turnover (g/d/kg)						
	^{14}C			^3H			
	Foetuses	0 Dead	1 Dead	2 Dead	0 Dead	1 Dead	2 Dead
14		2.04		1.73	2.16		1.91
47		1.63		2.21	1.77		2.45
51		1.74			1.88		
234		4.46			4.76		
253		2.06	4.59		2.17	5.08	
24			1.63			1.74	
235			4.89	10.52		5.06	12.59
256			4.74		5.18		

Twin-pregnant ewes were starved for four days, and then 0, 1 or 2 foetuses were killed in utero by tightening nooses, which had been surgically placed around each umbilical cord.

Glucose turnover was measured by the primed continuous infusion of [U- ^{14}C]- and [6- ^3H] glucose before, and 24 hours after foetal death.

The differences in recycling between groups were significant. Recycling in the ewes with two live lambs was 6.5% while in ewes with two dead lambs it was 12%. This could imply that the ewe was then using C_3 compounds which were previously metabolized by the fetuses.

Thus killing the fetuses raised glucose turnover, and increased the amount of recycling.

A summary of glucose turnover rates in all sheep is shown in Table 20.

9.3 HEPATOCYTE GLUCOSE PRODUCTION:

The hepatocyte glucose production rates expressed as $\mu\text{mol/g/30 min}$ above basal, are shown in Table 30.

No difference was found in production rates between groups with one or both lambs alive, and so the results of these groups were pooled. The rates of glucose production were lower in the ewes with live lambs, and these differences were significant with alanine, glycerol, dbcAMP, and with all substrates in the presence of 10^{-8} M glucagon.

Hepatocytes from ewes with live lambs did not respond to the presence of glucagon to the same extent as those with dead lambs.

The effect of the substrates is shown in Table 31. Propionate and lactate gave the highest rates. The effect of glucagon was most on the hepatocytes from ewes with dead lambs. Dibutyryl cyclic AMP is a derivative of cAMP and mediates the effect of glucagon on gluconeogenesis (Jost and Rickenberg, 1971) and was included as a measure of the integrity of glucagon receptors on the hepatocytes. If the receptors are intact and the adenyl cyclase functional, cAMP and glucagon should give the same result. In both groups the rates of production with dbcAMP were no different to those with glucagon.

TABLE 30: Effect of Killing Foetuses In Utero on Hepatocyte Glucose Production.

Foetuses	No.	gn + B	Glucose Production ($\mu\text{mol/g/30 min}$ above basal)									
			P	gn + P	L	gn + L	A	gn + A	Gc	gn. + Gc	dbcAMP	P + dbcAMP
0 Dead	2	0.85 ± 1.77	4.5 ± 4.3	4.8 ± 0.6	3.6 ± 4.2	6.2 ± 3.5	0.4 ± 0.1	3.1 ± 4.5	1.6 ± 0.6	0.7 ± 5.0	1.8 ± 2.7	6.8 ± 0.4
1 Dead	3	-0.1 ± 1.8	6.5 ± 7.6	4.5 ± 2.2	4.8 ± 6.6	2.5 ± 3.5	-0.6 ± 1.3	0.0 ± 3.8	-2.4 ± 3.4	-4.2 ± 14.5	-2.5 ± 4.9	5.1 ± 7.9
(pooled) 0/1 Dead	5	0.3 ^a ± 1.6	5.7 ± 5.9	4.6 ^a ± 1.6	4.3 ± 5.2	4.0 ^a ± 3.6	-0.2 ^a ± 1.1	1.3 ^a ± 3.9	-0.8 ^a ± 3.3	-2.3 ^a ± 10.9	-0.8 ^a ± 4.4	5.8 ± 5.7
2 Dead	3	22.4 ^b ± 13.3	16.2 ± 8.5	31.9 ^b ± 17.4	16.8 ± 6.3	22.7 ^b ± 8.8	16.9 ^b ± 8.5	21.8 ^b ± 11.5	9.7 ^b ± 5.8	14.4 ^b ± 6.3	9.1 ^b ± 3.7	30.1 ± 32.7

Mean \pm S.D.

Means with different superscripts differ significantly at 5% level, based on Wilcoxon's two-sample rank test.

Twin-pregnant ewes were starved for four days, and then 0, 1 or 2 foetuses were killed in utero by tightening nooses which had been surgically placed around each umbilical cord. The ewes were slaughtered after 24 hours.

Hepatocytes were isolated from the maternal caudate lobe, by perfusion with collagenase, and incubated for 30 min with substrate (10 mM) and glucagon (10^{-8} M).

B - Basal P - Propionate L - Lactate A - Alanine
Gc - Glycerol dbcAMP - dibutyl cyclic AMP gn - glucagon

TABLE 31: Effect of Substrate on Hepatocyte Glucose Production After Killing Foetuses In Utero.

Rank	Foetuses		
	0 Dead	1 Dead	2 Dead
Lowest			
1	B	dbcAMP	B
2	B + gn	Gc	dbcAMP
3	A	A	Gc
4	Gc + gn	B	Gc + gn
5	Gc	B + gn	A
6	dbcAMP	A + gn	P
7	L	Gc + gn	P + dbcAMP
8	A + gn	P + dbcAMP	L
9	P	L	B + gn
10	P + gn	L + gn	A + gn
11	L + gn	P	L + gn
12	P + dbcAMP	P + gn	P + gn
Highest			

There was no difference between substrates in the groups with 0 or one dead lambs, but all were significantly different from basal in the group with two dead lambs based on the non-parametric Chi-squared test ($p < 0.05$).

Twin-pregnant ewes were starved for four days, and then 0, 1 or 2 foetuses were killed in utero.

Hepatocytes were isolated from the maternal caudate lobe 24 hours later, and incubated with substrate (10 mM) and glucagon (10^{-8} M).

A - Alanine B - Basal dbcAMP - dibutyryl cyclic AMP
 Gc - Glycerol L - Lactate P - Propionate
 gn - glucagon

This implies that the lack of stimulation by glucagon on hepatocytes from ewes with live lambs is not due to the receptor mechanism.

9.4 SUMMARY:

Killing the foetuses in utero of ewes starved for four days, raised plasma glucose, reduced plasma acetoacetate, increased glucose turnover rates and recycling rates and raised the gluconeogenic potential of the hepatocytes. This suggests that in starvation the foetuses or foeto-placental unit influence maternal carbohydrate metabolism by restraining maternal hepatic glucose production.

CHAPTER 5

DISCUSSION

1. GLUCOSE METABOLISM OF FED EWES:1.1 GLUCOSE TOLERANCE AND INSULIN RESISTANCE:

The glucose tolerance test is a relatively simple technique for assessing an animal's ability to maintain glucose homeostasis, and the results of duplicate experiments show that it has good repeatability (Fig. 13). The test has been used in sheep to determine the effect of diet (Reid, 1958), fasting (Boda, 1964) and ketosis and OPT (Reid, 1960a) on glucose utilization, but it has not been used diagnostically, as in non-ruminants, to detect disorders of carbohydrate metabolism (Lundbaek, 1962; Horton *et al.*, 1968). Since hypoglycaemia is the main metabolic lesion in OPT and represents a loss of glucose homeostasis, it was hypothesized that the glucose tolerance test may provide criteria, prior to inducing the disease, for predicting a ewe's susceptibility to it.

Table 8 shows that preinjection glucose values were similar in all groups, and suggests that pregnancy per se does not alter plasma glucose concentration. However plasma insulin differed. The concentrations in non-pregnant sheep, at 10-31 $\mu\text{U/ml}$ were the same range as those reported by others for sheep fed ad lib (Bassett *et al.*, 1971), while the values for pregnant ewes were lower, and this seems to be a feature of late gestation (Hove and Blom, 1976).

Glucose tolerance, measured as $T_{\frac{1}{2}}$, was 36-40 minutes for non-pregnant ewes and was similar to that measured in ewes on lucerne roughage, by Reid (1958). The value for NS-L ewes was also within this range and so intolerance is not a feature of normal

pregnancy in sheep. However, in some ewes, the S-L and NS-D groups, the tolerance was impaired. The lower tolerance may be due to a slower uptake of glucose or to a continued endogenous production. However the effect is most likely to be due to utilization, as infusion of glucose generally suppresses gluconeogenesis (Judson and Leng, 1973a).

The diagnostic value of the test is increased when the insulin response is measured and, as shown in Fig. 14 the response varied between animals. Generally the levels peaked after 10 minutes and had returned to normal after two hours. They were thus similar to the responses observed in wethers (Boda, 1964).

Insulin kinetic models have shown that the rate at which glucose is utilized is related to insulin concentration in an extravascular compartment, rather than plasma, and represents the fluid in contact with the cells (Sherwin et al., 1974). From the extravascular insulin values in Table 8, it is apparent that, in sheep fed once daily, more insulin was produced to achieve the same rate of glucose disappearance. This implies that the cells were less sensitive to insulin and this is reflected in a higher insulin resistance index. The I' of the pregnant ewes were similar to the non-pregnant ewes on the same feeding regime and differ therefore from the human, where in pregnancy more insulin is released in response to a glucose load to achieve the same rate of glucose disappearance (Spellacy and Goetz, 1963).

The rate of glucose disappearance is related to the insulin concentrations by the resistance index, R. Its reciprocal, K_{ins} , the sensitivity index was defined by Bergman et al. (1979) as the dependence of fractional glucose disappearance on plasma insulin, and in dogs was calculated to be $7.10^{-4} \text{ min}^{-1}/(\mu\text{U/ml})$. The

reciprocal gives a resistance index of 1429 $\mu\text{U}/\text{ml}\cdot\text{min}$, and is similar to the values calculated for non-pregnant sheep, fed hourly, and NS-L ewes (Table 8). The resistance values of sheep fed oncedaily were higher and suggest that frequency of feeding may alter insulin response.

Table 8 also shows that in late pregnancy, ewes susceptible to OPT have a significantly higher resistance to insulin than those not susceptible. The higher resistance may be due to a defect in insulin production, a defect in the periphery such as receptor mechanism, or to the presence of insulin antagonists. In humans placental lactogen increases the insulin response to glucose in subclinical diabetic patients (Kalkhoff et al., 1969) and so ovine placental lactogen may also act as an antagonist. A glucose clamp has been developed to differentiate between the effects. It can be used to independently measure insulin secretion by the pancreas and glucose utilization by peripheral tissues (De Fronzo et al., 1979). Insulin secretion, or beta-cell sensitivity to glucose is measured by 'clamping' glucose concentration at an elevated level, and measuring the glucose input necessary to maintain it. To measure tissue sensitivity to insulin, insulin levels are raised and maintained by a continuous infusion of insulin, and the glucose input necessary to maintain basal glucose concentrations are measured. By this technique the mechanism contributing to insulin resistance, and hence the defect in the glucose homeostatic mechanism could be determined.

Although the value of the insulin resistance index depends on several processes and its physiological significance is debatable, it does appear to have predictive value. The susceptibility of ewes to OPT could be determined prior to starvation. If found to be

repeatable in a wider series of experiments, the technique would allow for better experimental design in the research of OPT.

In conclusion, susceptibility to OPT in pregnant ewes could not be determined from glucose or insulin levels during feeding, but a glucose tolerance test showed that ewes susceptible to OPT had higher insulin resistance (Table 8). This poor control over glucose homeostasis may be a predisposing factor in the development of the disease.

1.2 GLUCOSE KINETICS OF FED TWIN-PREGNANT EWES:

While the tolerance studies were designed to examine the response of a system to a challenge, the kinetic experiments examined the normal steady state metabolism. The experiments were undertaken first, to develop a kinetic model of glucose metabolism in fed, pregnant ewes, and secondly to use the model to assess whether differences occur in the glucose kinetics of susceptible and non-susceptible ewes.

1.2.1 GLUCOSE TURNOVER:

Glucose turnover, as the area under the decay curve was 125 g/day, and when expressed per kilogram of animal liveweight, is comparable to the value reported by Bergman (1963). He obtained a value of 180 g/day, and both convert to 2.33 g/kg/day.

The turnover rate calculated with tritium was higher (138 g/day) as this tracer is lost more rapidly from the system, and some workers contend that it represents a 'true' rate of glucose turnover. The difference between tracers is considered to be due to recycling of products and precursors of glucose metabolism, such as lactate and alanine.

The decay curves were best explained by the sum of five exponential terms, and although required for a good fit, were often not widely separated in slope (Table 10).

1.2.2 GLUCOSE MODEL:

The five compartment model proposed to explain glucose kinetics in twin-pregnant sheep differs from the three compartment model of Kronfeld et al. (1971) for lactating cows, and the four compartment models of Horsfield et al. (1974) for cows, and Hodgson and Mellor (1977) for pregnant sheep.

Early sampling resulted in plasma being identified as a separate pool, compartment 1, while the sum of the first three compartments was equivalent in size to extracellular fluid (Table 13). Compartment 4 is of similar size when estimated by the two tracers and this suggests that the label is still on glucose although ^3H has a faster turnover time. It may therefore represent intracellular glucose with some products. Compartment 5 is too large to be solely glucose, and in accord with other proposed models, is considered to be products and precursors of glucose. For this reason the recycling flow was incorporated from compartment 5 to compartment 2, and represents label reincorporated into glucose (Fig. 16).

Dunn et al. (1967) showed that according to known biochemical pathways, the 6- ^3H of glucose should be removed during resynthesis of glucose from pyruvate. Consequently recycling of ^3H should be zero. However, in concert with Kronfeld (1977) some ^3H did recycle. It represented 3% of glucose turnover, and may be partly the result of label from $^3\text{H}_2\text{O}$ being reincorporated into glucose, as this occurs to a small extent in sheep (Judson and Leng, 1972).

The recycling of carbon, at 8%, was slightly higher than the 4% calculated by Brockman et al. (1975b) using similarly labelled glucose tracers, in non-pregnant sheep.

Although the main differences between tracers when fitted to the model separately were in the flow rates $L(2, 5)$ and $L(0, 5)$ (Table 14), allowing these rates to vary was not sufficient to fit the tracers simultaneously. The model, although sufficient to explain the tracer data separately appears too simple to explain recycling adequately. Incorporating more compartments into the recycling portion of the model may help to reconcile the inconsistencies present in the current model.

1.2.3 GLUCOSE KINETICS OF SUSCEPTIBLE AND NON-SUSCEPTIBLE EWES:

The data obtained from all ewes, when fed, were analysed in terms of the outcome after a period of starvation.

In the S-L and NS-L sheep, movement of the two tracers were similar between the first four compartments (Fig. 19) and the tracers were recycled to a similar extent. However, in the NS-D group large differences occurred between the tracers moving between compartments 3 and 4 (Fig.19c). This suggests that in this group the label may have no longer been on glucose. Catabolism may have occurred in compartment 3, and the label was therefore on products.

To determine the differences between groups, comparing the carbon data, Table 17 shows that the fractional flow rates between compartments 1, 2, 3 and 5 are similar for the S-L and NS-L groups; however the flow rates to compartment 4 are higher in NS-L.

The flow rates of the NS-D are lower than the other groups and this is especially so between compartments 3 and 4 and implies that in this group, compartment 4 is slower turning over. In addition, in the NS-D group, a larger proportion (14%) of the carbon is recycled, compared to the other groups (8%). This larger recycling fraction may account for the slower decline at the end of the decay curve (Fig. 18c). The tritium data follow the same trends as the carbon, between groups.

These results suggest that the fractional flow rates, when compared to S-L are higher in NS-L, and lower in the NS-D due to the larger recycling fraction. The main difference between the groups appears to be movement of the label between compartments 3 and 4. Since the foeto-placental unit has a substantial uptake and metabolism of glucose, it may be represented by one compartment, but to identify if this is so, the build up of tracer should be experimentally monitored in the foetus, and compared with the predicted build up by the model. That is, it would be necessary to get a submodel for the foetus and placenta, and to study how well it represented compartment 4.

Before a valid physiological interpretation can be given to the model, it will be necessary to identify all the compartments. This is difficult with glucose as it is rapidly catabolized. However the build up could be monitored on major products, as has been done in rats (Baker and Huebotter, 1972), or by disturbing particular areas of metabolism and studying the effect on the model.

With the current data, no differences were shown in the kinetics of susceptible and non-susceptible ewes; however ewes in which the lambs died, on starvation, had smaller fractional flow rates, different tracer movements between compartments 3 and 4, and a

larger fraction recycling. Further experimentation is necessary to identify the compartments.

2. GLUCOSE METABOLISM OF STARVED AND TOXAEMIC EWES:

2.1 BLOOD METABOLITES:

Metabolites were measured throughout starvation as a guide to the physiological changes, to monitor the pathogenesis of OPT and, finally, to assess the degree of toxaemia at the time of slaughter.

2.1.1 CHANGES IN KETOTIC EWES:

Metabolite concentrations were measured prior to starvation (day 0, Tables 3 and 4) to provide control values, and daily throughout the starvation period.

In contrast to the non-pregnant ewes, where plasma glucose did not alter during 10 days of starvation (Table 6), the concentrations in pregnant ewes fell rapidly (Fig. 5) and all groups were hypoglycaemic (less than 30 mg/dl) after three days. This was caused by utilization exceeding production. After this time, the levels rose, and this occurrence has been noted by others (Procos, 1962b). The reason for this rise may be an increase in glucose production, as has been observed in dogs during insulin-induced hypoglycaemia (Sacca et al., 1979) or it may be due to conservation measures which further reduce glucose utilization. One example is the decreased foetal uptake of glucose that occurs on maternal starvation (Simmons et al., 1974).

Hyperketonaemia also occurred early in starvation. Ketones are produced when acetyl CoA is only partially oxidized, following

fat mobilization. Plasma acetoacetate increased four-fold after the first day of starvation and reflects very rapid production rates. The rate of increase of both acetoacetate and 3-hydroxybutyrate concentrations slowed down after day five of starvation (Figs 6 and 7). This indicates either that the utilization had increased (by increased oxidation or excretion) or that production had decreased.

Plasma free fatty acids were only measured in the 1977 experiments. A rapid increase was recorded over the first three days, after which the levels plateaued in the ketotic ewes with live lambs, and decreased in those with dead lambs (Fig. 8). High levels are caused mainly by lipolysis, or fat mobilization (Annison, 1960). The plateau represents a new steady state where the rate of utilization equals the production rate.

Urea and creatinine are two end-products of protein metabolism. Urea, a freely permeable molecule in the kidney, moves by passive diffusion along the concentration gradient, and is therefore excreted in proportion to its concentration, while the clearance of creatine (which is converted to the anhydride creatinine before excretion) increases sharply with increasing plasma concentrations (Osbaldeston, 1971). The plasma concentrations are normally maintained in a narrow range, even with increased protein catabolism, and elevated plasma or serum concentrations therefore indicate a defect in excretion. Urea concentrations increased gradually during starvation (Fig. 9) and indicated that there was an increase in protein catabolism. In 1977 the levels in ketotic ewes with dead lambs exceeded 80 mg/dl and indicated renal failure. Creatinine levels less than 3 mg/dl are considered normal but even at 1.5 mg/dl,

glomerular filtration rate may be reduced by 50% (Wolff, 1977). In both years the means of ketotic ewes with dead lambs (NS-D) exceeded 1.5 mg/dl and also indicated that renal failure had occurred in some of these ewes.

During kidney disease excessive amounts of inorganic phosphate are retained (Pitts, 1968) and serum levels greater than 9 mg/dl, as observed in the NS-D ewes, also indicate kidney damage (Fig. 11).

Since most metabolic processes only occur within a narrow pH range, the buffering ability of the plasma is important for normal metabolism. Total CO_2 is a measure of bicarbonate reserve, or acid-base balance and the normal range for sheep is 21-28 mM (Swenson, 1970). Although total CO_2 decreased in all groups on starvation (Fig. 11), NS-L ewes remained within the normal range while those of the NS-D were substantially reduced. This indicates the condition of metabolic acidosis which arises from the accumulation of ketoacids, when the kidney fails to excrete H^+ ions and resorb Na^+ or when plasma pH falls and cells metabolize anaerobically to produce lactic and pyruvic acids (Tasker, 1971).

The plasma proteins serve a variety of functions but an important one is to maintain osmotic pressure. On starvation the levels of both total protein and albumin increased by 10%, and may be the result of a change in the packed cell volume, as this also increased by about 10% (Tables 3 and 4). The osmotic pressure of the plasma therefore increased on starvation.

In conclusion, the changes in NS (or ketotic) ewes on starvation were a fall in plasma glucose and increases in ketones and free fatty acids. While the concentrations of urea, creatinine and inorganic phosphate increased, and total CO_2 decreased, to a small

extent in ewes with live lambs, the changes were severe in ewes with dead lambs, and indicated renal failure. No effective change occurred in plasma proteins, but a mild increase in packed cell volume occurred in all ewes on starvation.

2.1.2 PATHOGENESIS OF OPT:

Although hypoglycaemia is a symptom of OPT, the paradox, as has been noted by others (Procos and Gilchrist, 1966), was that the lowest glucose levels were often recorded in the NS group (Tables 3 and 4). This suggests that induction of symptoms may be related to glucose turnover or other metabolic changes rather than plasma glucose per se. A terminal hyperglycaemic (glucose greater than 80 mg/dl) developed in S-D ewes and may have been triggered before foetal death (O'Hara et al., 1975) although the mechanism is not known.

Like hypoglycaemia, hyperketonaemia is also associated with toxaemia, but in 1977 no differences were recorded in ketones between groups, and in 1978 up to day five of starvation, higher concentrations were recorded in the NS-D group (Table 4). This agrees with the findings of McClymont and Setchell (1955a) that ketosis per se does not predispose to toxaemia.

The highest plasma free fatty acids were recorded in the NS-L group, and there was no difference between the S-L and NS-D groups (Fig. 8). The appearance of symptoms does not therefore seem to be related to an under- or over-mobilization of fat.

The changes in creatinine, though mild initially, increased dramatically after day five in ewes with dead lambs, and to a smaller extent in S-L ewes (Fig. 10). While urea levels had a

different response pattern in the two years (Fig. 9), the highest concentrations in 1977 were in the NS-D and S-L groups. These results suggest renal failure in these groups. The high creatinine and apparently normal urea concentrations in 1978 (Table 4), may be the result of urea analyses not being done on all sheep.

Inorganic phosphate, the third indicator of renal function, exceeded 9 mg/dl in S-L and NS-D groups (Fig. 11a) and the total CO₂ concentrations showed that the degree of acidosis was similar in the two groups (Fig. 11b). These results are in contrast to Katz and Bergman (1966) who concluded that electrolyte changes and acidosis are mild in toxæmia; however their studies were undertaken on ewes with OPT but the degree of toxæmia in their ewes was not clarified. The results of the present study are in agreement with Parry and Taylor (1956) who found that renal function was impaired in toxæmic ewes, and that the severity increased with development of the disease.

The creatinine, urea, phosphate and total CO₂ results therefore all show that, as renal failure and metabolic acidosis had occurred in S-L and NS-D groups, the latter were not necessarily associated with OPT symptoms.

There was no significant difference in total plasma proteins, albumin or packed cell volume between the groups, although dehydration was more severe in ewes with dead lambs.

Thus, in agreement with O'Hara et al. (1975) the pathogenesis of OPT, with discrete sampling, cannot be distinguished on change of metabolites from changes in starved, ketotic ewes. Symptoms associated with renal failure occurred in ewes with OPT, and in ketotic ewes with dead lambs, and so although it may be linked to the disease, renal failure is not always accompanied by OPT symptoms.

The sequence of metabolite changes could be followed more accurately if the ewes were continuously sampled during starvation, as this would minimize any stress of sampling and would show any rapid changes of particular metabolites. Monitoring the well-being of the foetus throughout starvation would also give valuable information on events that might contribute to foetal death.

2.1.3 DEGREE OF TOXAEMIA AT TIME OF SLAUGHTER:

The ewes were slaughtered when they were recumbent with OPT, or at the end of 10 days starvation.

In both years, low plasma glucose and high ketones and free fatty acids were present in ewes with live lambs, regardless of OPT symptoms, and hyperglycaemia was present in ewes with dead lambs (Table 5). The concentrations of these metabolites were more dependent upon the state of the lamb than presence of OPT.

The symptoms of renal failure - high urea, creatinine and inorganic phosphate, and low total CO_2 , were present in toxaemic sheep, but were most severe in NS ewes with dead lambs. So in agreement with findings of the previous section, renal failure is not always associated with OPT symptoms and would seem to be a consequence, rather than an initiating factor, of the disease.

The plasma proteins did not differ between groups at the time of slaughter and would not seem to be affected by the disease. However turnover may have altered. Blood metabolite concentrations reflect net changes in production and utilization of a compound, and have limited value in describing the internal milieu. Kinetic

experiments may be necessary to detect changes in metabolism of ketotic and OPT ewes.

In conclusion, although there was large variability between ewes at the time of slaughter, and the numbers small, sheep with OPT symptoms could not be differentiated from NS sheep on any of the blood metabolites measured. Changes associated with renal failure were common to both groups while others, such as glucose, ketones and free fatty acids, were more sensitive to the state of the lambs than OPT symptoms.

2.2 LIVER FAT AND GLYCOGEN:

The normal liver fat levels of pregnant ewes were 2-4% fresh weight, and 20% in underfed and toxaemic ewes (Roderick et al., 1933; Ford, 1962). While toxaemic ewes in these studies tended to have high liver fat (Table 5), the results were not consistent and S-L ewes of 1978 had levels approaching normal (8%), and in both years NS ewes had the highest mean values. High fat content is a primary post-mortem criterion for the diagnosis of OPT. The low fat content of these OPT ewes suggests either some cases differed from the field condition of the disease, or alternatively liver fat is the result of some factor not related to OPT, in which case fat content is not a reliable diagnostic criterion. Thomson (unpub.); Reid (1960d); Parry (1950) and McClymont and Setchell (1955a), also observed cases of OPT with no visible fat content of the liver.

Glycogen levels have been reported as 3.7% of fresh weight in fed, pregnant ewes, 1.2% in ketotic ewes and 0.3% in toxaemic ewes (Roderick et al., 1933; Ford, 1962). The results of these experiments (Table 5) also show that the levels were ten-fold lower in toxaemic ewes (0.2%) compared to ketotic ewes (1.8%)

and indicate that OPT may be linked with abnormal carbohydrate function in the liver. No difference was shown between S-L or S-D ewes, so death of the lambs in susceptible ewes did not allow glycogen stores to be replenished.

Liver biopsies, if they could be done without trauma, prior to starvation, would indicate whether levels were normal initially, or whether a defect was obvious before starvation was imposed.

By comparison, the values for non-pregnant ewes have been reported as fat, 5.6% fresh weight (Snook, 1939) and glycogen 2.7% fresh weight in the fed animal and glycogen 1.1% in animals starved for 14 days (Sasaki et al., 1975). In this study, similar fat levels were recorded in fed ewes (Table 6). They doubled during starvation but remained lower than in the pregnant ewes. Glycogen values were variable between sheep, but did not alter on starvation.

The fat levels of the non-pregnant ewes were similar to the S ewes, while the glycogen levels were five times the S ewes. This evidence suggests that an impairment occurs in carbohydrate, rather than fat metabolism in OPT.

In summary, fat levels increased on starvation in the pregnant and non-pregnant ewes, but there was no difference between S and NS ewes. By contrast the glycogen levels were not altered on starvation in non-pregnant ewes but were reduced to 5% of normal in sheep with OPT.

2.3 ORGAN WEIGHTS:

The organs of ewes and foetuses were weighed post-mortem to compare weight changes in S and NS ewes and to relate these to differences in metabolism, as hypertrophy of endocrine organs suggests increased hormone secretion rates (Reid, 1960c).

Reid (1960c) noted that the adrenals of ewes with OPT were enlarged (6.7 g) compared to the non-pregnant ewes (3.8 g). However this study shows that the adrenals of ketotic ewes are also enlarged (Table 7), and therefore no difference occurs between ketotic and toxaemic ewes. Hypertrophy was most pronounced in ewes with dead lambs. This may indicate a larger response to stress in these animals. Adrenal size, therefore, is not related to OPT symptoms.

The NS-D group had significantly larger foetal livers and maternal liver, kidney and thyroids (Table 7). Liver weights normally fall in late pregnancy and increase in lactation (Fell et al., 1972). The mean liver weight of the NS-D ewes was 20% higher than NS-L and may indicate altered liver metabolism. The enlarged kidneys may be the result of renal failure which occurred in this group, as they often appeared pale and swollen. The thyroid gland secretes hormones which regulate metabolic rate, and the enlarged thyroids of the NS-D group suggest higher metabolic rates, and may account for the larger lambs which were found both years in this group.

In summary, no significant differences were observed in foetal or maternal organ weights between S and NS ewes. Ewes with dead lambs had large adrenals, while NS ewes with dead lambs had larger liver, kidneys and thyroid glands, and heavier foetuses. Considered together, these changes suggest that there may have been an increased metabolic rate in this group.

2.4 GLUCOSE TURNOVER:

2.4.1 NON-PREGNANT EWES:

The value of glucose turnover in fed non-pregnant ewes, measured by primed continuous infusion, was 2.2 g/kg/day (Table 18) and is comparable to those reported by Leng (1970) for sheep fed a lucerne diet once daily.

On starvation, the glucose levels did not alter, but glucose turnover fell significantly (Table 18), and, as noted by Steel and Leng (1973a), a basal rate was apparently reached by four days, as only a slight reduction in turnover occurred thereafter. The constant plasma glucose during the fall in turnover implies that production and utilization rates were reduced simultaneously.

Recycling, as the difference between ^{14}C and ^3H tracers, was not detected at any stage during fasting (Table 18), and differs therefore from the results of Brockman et al. (1975b), where recycling was 4-5% in feeding and fasting. However the plasma glucose levels of their sheep were considerably lower (45-36 mg/dl) than those of this study, and the results may be influenced by the feed composition.

In conclusion, non-pregnant sheep on fasting appear to maintain plasma glucose concentrations while reducing glucose turnover, and, having attained a new basal turnover rate, are able to maintain it for up to 10 days.

2.4.2 PREGNANT EWES:

The turnover of glucose was also measured in pregnant ewes, when recumbent with OPT, or at the end of 10 days of starvation. The ewes may not have been in steady state during the experiments; however the plateau specific activities were averaged over periods

of relatively constant plasma glucose. Radziuk et al. (1978) showed that infusion experiments did predict the rate of glucose appearance in conditions when the rate was changing.

Glucose turnover in S-L ewes was lower than in NS-L, even though the plasma glucose concentrations were similar (Table 19). More data are required on NS-L ewes; however a reduction in turnover in OPT was also observed by Kronfeld and Simesen (1961) where turnover was 1.23 g/kg/day in NS ewes and 0.81 g/kg/day in toxaemic sheep. Low rates of glucose production and utilization were therefore features of OPT, but only when the lambs were alive, as hyperglycaemia and high turnover rates occurred in those with dead lambs (Table 19). This evidence suggests that glucose turnover rates increase in some event linked with foetal death, and the hyperglycaemia shows that production then exceeded utilization. By implication, glucose production appears to be limited in ewes with live lambs. This theory is discussed with the hepatocyte results.

Although these results suggest that turnover is low in the terminal stages of OPT, Ford (1963b, c; 1965) on a small number of sheep, found no difference between the turnover of fed ewes and ewes with OPT. This suggests that turnover may be only slightly reduced in the early stages of the disease. To determine whether this is so, a continuous infusion experiment could be undertaken on ewes throughout starvation, to observe any changes with the progression of the disease.

Recycling, as the difference in turnover measured by [^{14}C] and [$6\text{-}^3\text{H}$]glucose, at 4% (Table 19), was similar in S and NS ewes, but the fraction was higher, and more variable in S-D ewes. This may be associated with the hyperglycaemia, as glucose conversion to

lactate increases with plasma glucose concentration (Reilly and Chandrasena, 1978).

In conclusion, S-L ewes had a low rate of glucose turnover; however the hyperglycaemia of S-D and NS-D ewes was accompanied by high turnover rates. The data suggest that turnover rates increase on death of the lambs.

2.5 HEPATOCYTE GLUCONEOGENIC POTENTIAL:

2.5.1 METHODOLOGY:

The preparation of fresh hepatocytes by enzymic perfusion of the liver was first described by Berry and Friend (1969) in rats. Subsequently the method was adapted for ruminants by Clark et al. (1976b) and several metabolic studies have been reported for sheep (Ash et al., 1976; Clark et al., 1976a,b; Ash and Pogson, 1977; Richardson and Livesey, 1979; Weekes et al., 1979; Morton et al., 1977).

The use of hepatocytes for studying liver metabolism has advantages over in vivo methods in that the effects of substrates and hormones can be measured independently of complex feedback controls, and has the advantage over organ perfusion in that several studies can be undertaken simultaneously. Since the cells are actually suspended in solution, better oxygenation is possible than with liver slices.

The morphology and metabolic viability of ovine hepatocytes, isolated enzymically, was studied by Ash and Pogson (1977) and the cells were considered to be intact, and had rates of gluconeogenesis comparable to those measured in vivo, and with perfused livers. Clark et al. (1976a) determined that the rates of glucose synthesis

by caudate cells were similar to those obtained from cells of the median lobe. Hence hepatocytes can be isolated from the caudate lobe that are viable, and have metabolic rates similar to those measured in vivo.

2. 5. 2 NON-PREGNANT EWES:

Hepatocytes from non-pregnant ewes synthesized glucose from propionate, alanine, lactate and glycerol (Table 21). Starving the sheep for up to 10 days did not appear to alter their gluconeogenic potential as the rates with each substrate approximated those reported by Ash and Pogson (1977), for fed sheep. That glucose synthesis occurred is shown by the greater glucose production rates in hepatocytes incubated with substrate compared to those incubated without substrate. As these studies measured net glucose production, it may be argued that glucose could be synthesized in the cell and stored as glycogen, rather than being released. However, Clark et al. (1976b) established that in lambs, even after glycogen stores were reduced, gluconeogenesis measured by the rate of glucose formation, was similar to the rate of isotopic precursor incorporation into glucose and glycogen. Unless the adult animal differs, therefore, glucose release reflects gluconeogenic rates.

The cells from starved sheep responded to hormones as the highest production rates were recorded with glucagon (Table 22). As the basal rate was stimulated by an amount similar to the substrates, this suggests that the cells responded to glucagon by increased glycogenolysis and gluconeogenesis. Considerable glycogen stores were found in these livers on post-mortem (Table 6).

Although the gluconeogenic potential does not appear to change on starvation, glucose turnover, and hence gluconeogenesis, decreases in vivo (Table 18). Production may be limited in vivo by the availability of precursors, such as propionate. However all the rate-limiting enzymes of glucose synthesis, except P-enolpyruvate carboxykinase increase on fasting (Filsell et al., 1969), and the lack of precursors may be compensated by increased enzyme activity. The in vitro results suggest there is no overall change in the maximum gluconeogenic potential and imply that the enzyme changes alter the efficiency rather than the capacity of the system. More comparative data are required from fed, non-pregnant ewes.

The variability of the production rates, although partly a feature of this technique, may also reflect genuine differences between sheep in their ability to adapt to fasting, and therefore differences in their ability to maintain glucose homeostasis.

In conclusion, the results suggest that although responses vary, the gluconeogenic capacity of hepatocytes from non-pregnant ewes is not altered after 10 days fasting.

2.5.3 PREGNANT EWES:

The gluconeogenic potential of ketotic and toxaemic ewes was assessed, and the results of two years show, that in the presence of adequate substrate, the production rates of susceptible ewes are significantly lower than non-susceptible ewes (Table 23). The rates of glucose synthesis in the presence of substrates were no different from basal (Table 25) and the rates were not stimulated by glucagon (Table 24). This evidence suggests that liver cells from toxaemic ewes have a low ability to synthesize glucose, and do not

respond to hormonal stimulation. The gluconeogenic capacity was not increased in S ewes with dead lambs, and so the defect seems to be a feature of OPT which is not linked to foetal well-being.

Possible reasons for the low synthetic rates in these ewes could be a failure to take up substrate, and this needs to be examined with radioactive precursors. Alternatively the defect may be in the synthesis and release of glucose, and involve enzymes. The activities of the key gluconeogenic enzymes have not been measured in OPT; however production rates with glycerol (which is incorporated by a different pathway, Fig.1), were no higher, so the deficiency may lie with fructose-1,6-biphosphatase or glucose-6-phosphatase. The low gluconeogenic rates may be the result of energy deficiency, as Gallagher (1959) showed that livers from OPT sheep were unable to oxidize long chain fatty acids. In rats, hypoglycaemia was due to substrate availability and a lack of energy from fat oxidation (Ferre *et al.*, 1978). A stress syndrome in chickens, which is also characterized by low rates of gluconeogenesis, can be prevented by biotin, which influences the activity of pyruvate carboxylase (Balnave *et al.*, 1977), and a coenzyme deficiency may be implicated in OPT. Further work is indicated in the fields of hormonal status and gluconeogenic enzyme activity in ketotic and OPT ewes.

The gluconeogenic capacity of NS-L ewes was less than non-pregnant ewes starved for the same period of time (Table 26). This suggests that despite the higher demand for glucose by the pregnant ewe, they appear to have a lower gluconeogenic capacity measured in vitro. The capacity may be limited by enzyme activity, as P-enolpyruvate carboxykinase activity is not increased in starvation

and pyruvate carboxylase increases to a greater extent in non-pregnant ewes (Filsell et al., 1969). Either of these enzymes, therefore, could limit production in the ketotic ewe.

However cells from NS ewes with dead lambs synthesized glucose at a significantly higher rate than those with live lambs (Table 23), and showed greater stimulation by glucagon (Table 24) although this may reflect glycogenolysis from the larger glycogen stores (Table 5). The high rates of glucose production suggest that either these ewes produced at an inherently higher rate throughout starvation, or that the gluconeogenic capacity increased with foetal death. Since the two events may be linked to a common cause, or may be related directly, experiments were undertaken to study the direct effect of foetal death on maternal gluconeogenic capacity, and these results are discussed in Section 3.

In conclusion, toxæmic ewes have a low gluconeogenic capacity which suggests that OPT may be related to an inability to produce glucose. The capacity of non-susceptible ewes, with live lambs, was slightly lower than starved non-pregnant ewes, and was significantly lower than ewes with dead lambs. This evidence suggests that the presence of the live lambs may inhibit glucose production in the fasted, pregnant ewe.

2.6 RELATIONSHIP OF IN VITRO AND IN VIVO RESULTS:

Fig. 21 shows the relationship of the in vitro and in vivo results. The gluconeogenic capacity of the S-L ewes is consistent with the low glucose turnover and low glycogen reserves of the liver (Table 5). The higher turnover rates of the NS-L ewes, and very high turnovers of the NS-D ewes are associated with increased gluconeogenic capacity (Tables 19 and 23). However the S-D ewes

recorded high turnover rates, but had low gluconeogenic capacities (Fig. 21). This suggests that glucose may be produced in vivo from a source other than the liver. One possibility is renal gluconeogenesis. The activities of some gluconeogenic enzymes increase in the kidney of fasted pregnant ewes (Filsell et al., 1969). However studies on hypoglycaemic (Kaufman and Bergman, 1974) and acidotic sheep (McIntosh et al., 1973) showed that production is small and is unlikely to exceed 6 g/day, and would not therefore account for the high production rates. Alternatively, the glucose may result from breakdown of glycogen stores as the S-D ewes had low liver glycogen levels (2 mg/g) on post-mortem compared with NS-D ewes (18 mg/g) (Table 5). A glycogen store of 18 mg/g, if released at 7 g/kg/day (Table 19) would last only several hours, and toxaemic ewes generally die within a short period of developing hyperglycaemia (Reid, 1960d). In bovine ketosis the glycogen stores are also depleted and Kronfeld et al. (1960) suggested that sympatho-adrenal activity, glucagon secretion or hypoglycaemia may act as initiators of rapid glycogenolysis. However the liver glycogen levels of S-L ewes, before foetal death, were no higher, and would tend to discount this theory.

An alternative explanation is that the cells may not represent the in vivo situation, as a result of the disease and cell preparation. However the low glycogen levels in these ewes also suggest that glucose metabolism is abnormal.

In conclusion, S-D ewes have high glucose turnover, but exhibit a low hepatic gluconeogenic capacity. In vivo incorporation studies may be necessary to test the in vitro results.

3. GLUCOSE METABOLISM OF PREGNANT EWES BEFORE AND AFTER FOETAL DEATH IN UTERO.

It is generally thought that the hypoglycaemia of starved, pregnant ewes is due to a continued high foetal demand for glucose, and insufficient glucose production due to limited precursor availability (Reid, 1968). However foetal uptake does fall during starvation in response to the lower materno-foetal gradient (Simmons *et al.*, 1974). This suggests that the hypoglycaemia, therefore, may not be a result of high foetal demand, but may be due to limited glucose production. The experiments described earlier in this study showed that plasma glucose, glucose turnover and hepatocyte glucose production were lower in starved ewes with live foetuses (Tables 20 and 26). This suggested that the foeto-placental unit may be limiting maternal glucose production, and these experiments were designed to examine this theory.

Tables 27 and 28 show that deliberately killing the foetuses, after four days of starvation, increased plasma glucose significantly, and glucose turnover. These increases may have resulted from increased availability of precursors; however *in vitro* experiments with hepatocytes showed that cells from ewes with live lambs, in the presence of adequate substrate, had lower glucose production rates (Table 30). This table also shows that the rates of glucose production with dbcAMP were similar to the corresponding rates with glucagon. This suggests that the hormone receptors were intact and that the defect was within the gluconeogenic mechanism. Killing the foetuses therefore raised the gluconeogenic capacity of the maternal hepatocytes.

Hepatic glucose production thus appears to be restrained in starved ewes with live lambs by some foeto-placental factor. The effect may be hormonal and regulate production through enzyme

activity. A hormone present in high concentrations in late pregnancy, and which is known to affect maternal glucose metabolism, is ovine placental lactogen (OPL). In addition, the levels are higher in twin-pregnancies (Handwerger et al., 1977) and, in humans, are increased during starvation (Kim and Felig, 1971). Infusion of OPL in both pregnant and non-pregnant ewes lowered plasma glucose, and increased insulin (Handwerger et al., 1976), and so it may affect glucose production directly, or indirectly through insulin. Any direct effect should be demonstrable by incubating normal hepatocytes with OPL and measuring the effect on glucose production. Insulin is normally decreased in starvation (Bassett and Madill, 1974), and gluconeogenesis is favoured. However, if the insulin levels were increased through pancreatic stimulation from OPL, glucose production would be reduced. OPL levels would be expected to be high in starved twin-pregnant ewes in late gestation and may therefore affect glucose production.

Recycling increased significantly on foetal death (Table 28) and may be a consequence of the hyperglycaemia, as this increases the conversion of glucose to lactate (Reilly and Chandrasena, 1978). Alternatively, as recycling (the difference between $[U-^{14}C]$ and $[6-^3H]$ glucose) reflects the activities of enzymes in the dicarboxylic acid shuttle (Dunn et al., 1977), the increase may be due to an increase in the activities of pyruvate carboxylase or P-enolpyruvate carboxykinase.

Further studies are indicated to determine the nature of the gluconeogenic suppression in the pregnant ewe during starvation, and these include monitoring OPL levels, measuring the activities of gluconeogenic enzymes and determining the insulin/glucagon ratio prior to, and following foetal deaths.

In conclusion, killing the foetuses in utero, of ewes starved for four days, caused an increase in plasma glucose, glucose turnover and gluconeogenic capacity of the hepatocytes. This suggests that the hypoglycaemia of starved pregnant ewes is due to inhibition of glucose production by some foeto-placental factor.

CHAPTER 6

SUMMARY

In this study glucose metabolism was studied in fed and starved pregnant ewes in relation to the development of ovine pregnancy toxæmia. The ewes were classified, after a period of starvation, as susceptible (S) if they became recumbent with OPT, otherwise non-susceptible (NS). They were further grouped according to whether the lambs were dead or alive at the time the ewes were slaughtered.

Glucose tolerance tests in fed ewes showed that those susceptible to OPT had higher insulin resistance than those not susceptible. This phenomenon may have importance in the research field for screening sheep before inducing the disease, and it also suggests that poor control over glucose homeostasis may be a predisposing factor in the development of OPT.

The glucose kinetics of susceptible and non-susceptible ewes were compared with the use of a 5-compartment model, which was developed following the injection of [U- ^{14}C]- and [6- ^3H] glucose to explain glucose kinetics in the twin-pregnant ewe. The parameters of the model for ewes with live lambs were similar in S and NS groups, but NS ewes with dead lambs had smaller fractional flow rates, and a larger fraction recycling which resulted in a slower decay curve. In addition, tracer movement between compartments 3 and 4 differed in this group, although the composition of these compartments awaits further experimentation.

On starvation, at about 130 days gestation, plasma glucose decreased, while ketones and free fatty acids increased in all groups of ewes. Symptoms of renal failure - increased urea,

creatinine and inorganic phosphate, and decreased total CO_2 were seen in toxaemic ewes, and NS ewes with dead lambs. At the time of slaughter, ewes recumbent with OPT could not be differentiated biochemically from ketotic ewes. Changes associated with renal failure were common to S and NS ewes, and this suggests that it may be a consequence rather than an initiating factor of the disease. Concentrations of other metabolites, such as glucose, ketones and free fatty acids were more sensitive to the state of the lambs than to OPT.

On starvation, liver fat increased in non-pregnant and pregnant ewes, but to no greater extent in sheep with OPT. Liver glycogen levels of non-pregnant ewes did not change on starvation, while the levels decreased in pregnant sheep. Glycogen of toxaemic ewes was 10% the level of ketotic ewes, and 5% of normal.

No differences were found in the adrenal weights of S and NS ewes, but NS ewes with dead lambs had larger liver, kidneys and thyroids suggesting that there was a higher metabolic rate in these sheep.

Plasma glucose of non-pregnant ewes did not change during starvation, but glucose turnover decreased significantly after four days. No further decrease occurred after 10 days fasting. However the gluconeogenic potential assessed in vitro by isolated hepatocytes was not altered after 10 days of starvation, when compared to reported rates for fed non-pregnant ewes (Ash and Pogson, 1977). Production may be limited in vivo by precursor availability.

The glucose turnover of S ewes with live lambs, was significantly lower than S or NS ewes with dead lambs, and implied that the hyperglycaemia of ewes with dead lambs was accompanied by increased glucose turnover.

The gluconeogenic capacity of the liver was assessed in vitro by incubating hepatocytes with substrates, and glucagon. Susceptible ewes had a significantly lower rate of glucose production and the rates were not stimulated by glucagon. These results were obtained when the foetuses were dead or alive, and suggested that low glucose production may be a feature of terminal toxemia in ewes. However, in S ewes with dead lambs the in vitro results could not account for the glucose turnover rate recorded in vivo, and so the glucose may have been produced from a source other than the liver, or the low in vitro rates may have been a consequence of the technique. Glucose production in toxemic ewes may be limited by an energy deficiency, as it has been shown that livers from toxemic sheep are unable to oxidize long chain fatty acids (Gallagher, 1959).

The glucose production rates of hepatocytes from ketotic ewes with live lambs (NS-L), though significantly higher than toxemic ewes, were lower than those of starved non-pregnant ewes, and significantly lower than NS ewes with dead lambs. These results suggested that the live foetus was restraining maternal glucose production.

Killing the foetus in utero of ewes starved for four days, increased the plasma glucose, glucose turnover and hepatocyte gluconeogenic potential and provided direct evidence of the inhibition of glucose production in starved pregnant ewes. One factor involved in the inhibition may be ovine placental lactogen, as it is present in high concentrations in late pregnancy, and is known to alter maternal carbohydrate metabolism (Handwerger et al., 1976). The results suggested that the hypoglycaemia of starved pregnant ewes may be due to inhibition of glucose production by some foeto-placental factor.

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REFERENCES

- ALBANO, J.; EKINS, R.P. 1970. The attainment of high sensitivity and precision in radioimmunoassay techniques as exemplified in a simple assay of serum insulin, p.491-513. In: International Atomic Energy Agency. In vitro procedures with radioisotopes in medicine. Vienna: IAEA.
- ALEXANDER, D.P.; BRITTON, H.G.; COHEN, N.M.; NIXON, D.A. 1969. Foetal metabolism, p.95-113. In: Wolstenholme, G.E.W.; O'Connor, M. (Ciba Foundation) Foetal autonomy. London: Churchill.
- ANNISON, E.F. 1960. Plasma non-esterified fatty acids in sheep. Australian Journal of Agricultural Research 11: 58-64.
- ANNISON, E.F.; BROWN, R.E.; LENG, R.A.; LINDSAY, D.B.; WEST, C.E. 1967. Rates of entry and oxidation of acetate, glucose, D- (-) - β - hydroxybutyrate, palmitate, oleate and stearate, and rates of production and oxidation of propionate and butyrate in fed and starved sheep. Biochemical Journal 104: 135-147.
- ANNISON, E.F.; LINDSAY, D.B.; WHITE, R.R. 1963. Metabolic interrelations of glucose and lactate in sheep. Biochemical Journal 88: 243-248.
- ANNISON, E.F.; WHITE, R.R. 1961. Glucose utilization in sheep. Biochemical Journal 80: 162-169.
- ASH, R.; ELLIOTT, K.R.F.; POGSON, C.I. 1976. Preparation and utilization of isolated hepatocytes for the study of sheep liver metabolism. Proceedings of the Nutrition Society 35(1): 29A.
- ASH, R.; POGSON, C.I. 1977. Preparation and biochemical characterisation of isolated parenchymal cells from adult sheep liver. Biochimica et Biophysica Acta 496: 475-483.
- ATHANASIOU, V.N.; PHILLIPS, R.W. 1978. Effect of fasting on plasma metabolites and hormones in lactating dairy cows. American Journal of Veterinary Research 39: 957-960.
- BAILE, C.A. 1968. Regulation of feed intake in ruminants. Federation Proceedings. Federation of American Societies for Experimental Biology 27: 1361-1366.

- BAIRD, G.D.; HEITZMAN, R.J.; HIBBITT, K.G.; HUNTER, G.D. 1974. Clinical problems of preventive medicine. Bovine Ketosis: a review with recommendations for control and treatment. Part II. British Veterinary Journal 130: 318-326.
- BAKER, N.; HUEBOTTER, R.J. 1972. Compartmental and semi-compartmental approaches for measuring glucose carbon flux to fatty acids and other products in vivo. Journal of Lipid Research 13: 716-724.
- BALLARD, F.J.; HANSON, R.W.; KRONFELD, D.S. 1969. Gluconeogenesis and lipogenesis in tissue from ruminant and nonruminant animals. Federation Proceedings. Federation of American Societies for Experimental Biology 28: 218-231.
- BALLARD, F.J.; HANSON, R.W.; KRONFELD, D.S.; RAGGI, F. 1968. Metabolic changes in liver associated with spontaneous ketosis and starvation in cows. Journal of Nutrition 95: 160-172.
- BALLARD, F.J.; OLIVER, I.T. 1965. Carbohydrate metabolism in liver from foetal and neonatal sheep. Biochemical Journal 95: 191-200.
- BALNAVE, D.; WOLFENDEN, J.; BALL, F.M.; CUMMING, R.B.; LENG, R.A. 1977. Studies of fatty liver and kidney syndrome in chickens: dynamics of glucose metabolism. British Journal of Nutrition 38: 329-334.
- BASSETT, J.M. 1970. Metabolic effects of catecholamines in sheep. Australian Journal of Biological Sciences 23: 903-914.
- BASSETT, J.M. 1971. The effects of glucagon on plasma concentrations of insulin, growth hormone, glucose, and free fatty acids in sheep: comparison with the effects of catecholamines. Australian Journal of Biological Sciences 24: 311-320.
- BASSETT, J.M. 1972. Plasma glucagon concentrations in sheep: their regulation and relation to concentrations of insulin and growth hormone. Australian Journal of Biological Sciences 25: 1277-1287.
- BASSETT, J.M. 1975. Dietary and gastro-intestinal control of hormones regulating carbohydrate metabolism in ruminants, p. 383-398. In: McDonald, I.W.; Warner, A.C.I. Digestion and metabolism in the ruminant. (IV International Symposium on Ruminant Physiology) Armidale: University of New England.

- BASSETT, J.M. 1977. Glucagon, insulin and glucose homeostasis in the fetal lamb. Annales de Recherches Veterinaires 8(4): 362-373.
- BASSETT, J.M.; MADILL, D. 1974. The influence of maternal nutrition on plasma hormone and metabolite concentrations of foetal lambs. Journal of Endocrinology 61: 465-477.
- BASSETT, J.M.; MILLS, S.C.; REID, R.L. 1966. The influence of cortisol on glucose utilization in sheep. Metabolism: Clinical and Experimental 15: 922-932.
- BASSETT, J.M.; WALLACE, A.L.C. 1966. Short-term effects of ovine growth hormone on plasma glucose, free fatty acids and ketones in sheep. Metabolism: Clinical and Experimental 15: 933-944.
- BASSETT, J.M.; WESTON, R.H.; HOGAN, J.P. 1971. Dietary regulation of plasma insulin and growth hormone concentrations in sheep. Australian Journal of Biological Sciences 24: 321-330.
- BATTAGLIA, F.C.; MESCHIA, G. 1973. Foetal metabolism and substrate utilization, p.382-397. In: Sir Joseph Barcroft Centenary Symposium. Foetal and neonatal physiology. (Proceedings of the Sir Joseph Barcroft Centenary Symposium at Cambridge 1972) London: Cambridge University Press.
- BAUR, H.; KASPEREK, S.; PFAFF, E. 1975. Criteria of viability of isolated liver cells. Hoppe-Seyler's Zeitschrift fur Physiologische Chemie 356: 827-838.
- BELL, J.P.; SALAMONSEN, L.A.; HOLLAND, G.W.; ESPINER, E.A.; BEAVEN, D.W.; HART, D.S. 1970. Autotransplantation of the pancreas in sheep: insulin secretion from the transplant. Journal of Endocrinology 48: 511-525.
- BERGMAN, E.N. 1963. Quantitative aspects of glucose metabolism in pregnant and nonpregnant sheep. American Journal of Physiology 204: 147-152.
- BERGMAN, E.N. 1964. Glucose turnover rates in pregnant and non-pregnant sheep. Nature 202: 1333.
- BERGMAN, E.N. 1968. Glycerol turnover in the nonpregnant and ketotic pregnant sheep. American Journal of Physiology 215: 865-873.

- BERGMAN, E.N. 1973. Glucose metabolism in ruminants as related to hypoglycemia and ketosis. Cornell Veterinarian 63: 341-382.
- BERGMAN, E.N.; HOGUE, D.E. 1967. Glucose turnover and oxidation rates in lactating sheep. American Journal of Physiology 213: 1378-1384.
- BERGMAN, E.N.; KATZ, M.L.; KAUFMAN, C.F. 1970. Quantitative aspects of hepatic and portal glucose metabolism and turnover in sheep. American Journal of Physiology 219: 785-793.
- BERGMAN, E.N.; KAUFMAN, C.F.; WOLFF, J.E.; WILLIAMS, H.H. 1974. Renal metabolism of amino acids and ammonia in fed and fasted pregnant sheep. American Journal of Physiology 226: 833-837.
- BERGMAN, E.N.; KON, K. 1964. Acetoacetate turnover and oxidation rates in ovine pregnancy ketosis. American Journal of Physiology 206: 449-452.
- BERGMAN, E.N.; STARR, D.J.; REULEIN, S.S. Jr. 1968. Glycerol metabolism and gluconeogenesis in the normal and hypoglycemic ketotic sheep. American Journal of Physiology 215: 874-880.
- BERGMAN, E.N.; IDER, Y.Z.; BOWDEN, C.R.; COBELLI, C. 1979. Quantitative estimation of insulin sensitivity. American Journal of Physiology 236: E667-E677.
- BERMAN, M. 1965. Compartmental analysis in kinetics, p.173-201. In: Stacy, R.W.; Waxman, B. Computers in biomedical research. Vol. II. New York: Academic Press.
- BERMAN, M. 1969. Kinetic modeling in physiology (Supplement). Federation of European Biochemical Societies, Letters 2: S56-S57.
- BERMAN, M.; SCHOENFELD, R. 1956. Invariants in experimental data on linear kinetics and the formulation of models. Journal of Applied Physics 27: 1361-1370.
- BERMAN, M.; SHAHN, E.; WEISS, M.F. 1962a. The routine fitting of kinetic data to models: a mathematical formalism for digital computers. Biophysical Journal 2: 275-287.
- BERMAN, M.; WEISS, M.F. 1974. Users manual for SAAM (Simulation, Analysis and Modeling) Version SAAM25. Bethesda: National Institutes of Health. (US Department of Health, Education and Welfare.)

- BERMAN, M.; WEISS, M.F.; SHAHN, E. 1962b. Some formal approaches to the analysis of kinetic data in terms of linear compartmental systems. Biophysical Journal 2: 289-316.
- BERRY, M.N. 1976. The development of techniques for the preparation of hepatic parenchymal cell suspensions - a history and rationale, p.131-138. In: Tager, J.M.; Soling, H.D.; Williamson, J.R. Use of isolated liver cells and kidney tubules in metabolic studies. Amsterdam: North-Holland Publishing Co.
- BERRY, M.N.; FRIEND, D.S. 1969. High-yield preparation of isolated rat liver parenchymal cells. A biochemical and fine structural study. Journal of Cell Biology 43: 506-520.
- BLAIR, A.; SEGAL, S. 1960. The isolation of blood glucose as potassium gluconate. Journal of Laboratory and Clinical Medicine 55: 959-964.
- BLAXTER, K.L. 1957. The effects of defective nutrition during pregnancy in farm livestock. Proceedings of the Nutrition Society 16: 52-58.
- BLOM, A.K.; HOVE, K.; NEDKVITNE, J.J. 1976. Plasma insulin and growth hormone concentrations in pregnant sheep II: post-absorptive levels in mid and late pregnancy. Acta Endocrinologica 82: 553-560.
- BLOOM, S.R.; EDWARDS, A.V. 1975. The release of pancreatic glucagon and inhibition of insulin in response to stimulation of the sympathetic innervation. Journal of Physiology (London) 253: 157-173.
- BODA, J.M. 1964. Effect of fast and hexose injection on serum insulin concentrations of sheep. American Journal of Physiology 206: 419-424.
- BONE, A.J.; HOWELL, S.L. 1977. Alterations in regulation of insulin biosynthesis in pregnancy and starvation studied in isolated rat Islets of Langerhans. Biochemical Journal 166: 501-507.
- BOYD, R.D.H.; MORRISS, F.H. Jr.; MESCHIA, G.; MAKOWSKI, E.L.; BATTAGLIA, F.C. 1973. Growth of glucose and oxygen uptakes by fetuses of fed and starved ewes. American Journal of Physiology 225: 897-902.

- BRITTON, H. G. ; HUGGETT, A. ST. G. ; NIXON, D. A. 1967. Carbohydrate metabolism in the sheep placenta. Biochemica et Biophysica Acta 136: 426-440.
- BROCKMAN, R. P. 1976. Effects of glucagon and insulin on lipolysis and ketogenesis in sheep. Canadian Journal of Comparative Medicine 40: 166-170.
- BROCKMAN, R. P. 1977. Glucose-glucagon relationships in adult sheep. Canadian Journal of Comparative Medicine 41: 95-97.
- BROCKMAN, R. P. 1978. Roles of glucagon and insulin in the regulation of metabolism in ruminants - a review. Canadian Veterinary Journal 19: 55-62.
- BROCKMAN, R. P. 1979. Roles for insulin and glucagon in the development of ruminant ketosis - a review. Canadian Veterinary Journal 20: 121-126.
- BROCKMAN, R. P. ; BERGMAN, E. N. 1975a. Effect of glucagon on plasma alanine and glutamine metabolism and hepatic gluconeogenesis in sheep. American Journal of Physiology 228: 1627-1633.
- BROCKMAN, R. P. ; BERGMAN, E. N. 1975b. Quantitative aspects of insulin secretion and its hepatic and renal removal in sheep. American Journal of Physiology 229: 1338-1343.
- BROCKMAN, R. P. ; BERGMAN, E. N. ; JOO, P. K. ; MANNS, J. G. 1975a. Effects of glucagon and insulin on net hepatic metabolism of glucose precursors in sheep. American Journal of Physiology 229: 1344-1350.
- BROCKMAN, R. P. ; BERGMAN, E. N. ; POLLAK, W. L. BRONDUM, J. 1975b. Studies of glucose production in sheep using [6-³H] glucose and [U-¹⁴C] glucose. Canadian Journal of Physiology and Pharmacology 53: 1186-1189.
- BROCKMAN, R. P. ; JOHNSON, M. R. 1977. Evidence of a role for glucagon in regulating glucose and β -hydroxybutyrate metabolism in sheep. Canadian Journal of Animal Science 57: 177-180.
- BROCKMAN, R. P. ; MANNS, J. G. 1974. Effects of glucagon on activities of hepatic enzymes in sheep. Cornell Veterinarian 64: 217-224.
- BROWNELL, G. L. ; BERMAN, M. ; ROBERTSON, J. S. 1968. Nomenclature for tracer kinetics. International Journal of Applied Radiation and Isotopes 19: 249-262.

BURD, L.I.; JONES, M.D.Jr.; SIMMONS, M.A.; MAKOWSKI, E.L.; MESCHIA, G.; BATTAGLIA, F.C. 1975. Placental production and foetal utilisation of lactate and pyruvate. Nature 254: 710-711.

BURTIS, C.A.; TROUTT, H.F.; GOETSCH, G.D.; JACKSON, H.D. 1968. Effects of glucagon, glycerol, and insulin on phlorizin-induced ketosis in fasted, nonpregnant ewes. American Journal of Veterinary Research 29: 647-655.

CAMERON, H.S.; GOSS, H. 1940. Preparturient paresis in ewes. Journal of the American Veterinary Medical Association 96: 165-170.

CHAN, J.S.D.; ROBERTSON, H.A.; FRIESEN, H.G. 1978. Distribution of binding sites for ovine placental lactogen in the sheep. Endocrinology 102: 632-640.

CHASSON, A.L.; GRADY, H.J.; STANLEY, M.A. 1961. Determination of creatinine by means of automatic chemical analysis. American Journal of Clinical Pathology 35: 83-88.

CHRISTENSON, R.K.; PRIOR, R.L. 1978. Uterine blood flow and nutrient uptake during late gestation in ewes with different number of fetuses. Journal of Animal Science 46: 189-200.

CLARK, M.G.; FILSELL, O.H.; JARRETT, I.G. 1976a. Gluconeogenesis in isolated lamb liver cells, p. 398-401. In: Tager, J.M.; Soling, H.D.; Williamson, J.R. Use of isolated liver cells and kidney tubules in metabolic studies. Amsterdam: North-Holland Pub. Co.

CLARK, M.G.; FILSELL, O.H.; JARRETT, I.G. 1976b. Gluconeogenesis in isolated intact lamb liver cells. Effects of glucagon and butyrate. Biochemical Journal 156: 671-680.

CLARK, R. 1943. Domsiekte or pregnancy disease in sheep. IV. - the effect of obesity on the reaction of sheep to a sudden reduction in diet. Onderstepoort Journal of Veterinary Science and Animal Industry 18: 279-288.

CLARK, R.; GROENEWALD, J.W.; MALAN, J.R. 1943. Domsiekte or pregnancy disease in sheep - III. Onderstepoort Journal of Veterinary Science and Animal Industry 18: 263-278.

COOP, I.E. 1950. The effect of level of nutrition during pregnancy and during lactation on lamb and wool production of grazing sheep. Journal of Agricultural Science (Cambridge) 40: 311-340.

- CRANE, B.; LANE, C. 1977. Modified semiautomated method for free fatty acids in serum. Journal of Clinical Pathology 30: 754-757.
- CUNNINGHAM, N.F. 1962a. The insulin activity of bovine and ovine blood plasma. I. Biological assay of insulin using the isolated rat diaphragm. Journal of Endocrinology 25: 35-42.
- CUNNINGHAM, N.F. 1962b. The insulin activity of bovine and ovine blood plasma. II. The insulin-like action of extracts of bovine and ovine blood plasma. Journal of Endocrinology 25: 43-52.
- CUNNINGHAM, V.J.; HEATH, D.F. 1978. An interpretation of the intravenous glucose tolerance test in the light of recent findings on the kinetics of glucose and insulin in man. Clinical Science and Molecular Medicine 54: 161-173.
- DANIEL, R.R.; METZGER, B.E.; FREINKEL, N.; FALOONA, G.R.; UNGER, R.H.; NITZAN, M. 1974. Carbohydrate metabolism in pregnancy. XI. Response of plasma glucagon to overnight fast and oral glucose during normal pregnancy and in gestational diabetes. Diabetes 23: 771-776.
- DASH, J.A.; LINDSAY, D.B. 1967. Comparison of an immunoassay and bioassay of insulin in sheep plasma. Journal of Endocrinology 37: 119-128.
- DAVIES, P.J.; JOHNSTON, R.G.; ROSS, D.B. 1971. The influence of energy intake on plasma levels of glucose, non-esterified fatty acids and acetone in the pregnant ewe. Journal of Agricultural Science (Cambridge) 77: 261-265.
- DAVIS, G.B. 1974. A sheep mortality survey in Hawke's Bay. New Zealand Veterinary Journal 22: 39-42.
- DAVIS, S.L. 1972. Plasma levels of prolactin, growth hormone, and insulin in sheep following the infusion of arginine, leucine and phenylalanine. Endocrinology 91: 549-555.
- DAWES, G.S.; MOTT, J.C.; SHELLEY, H.J. 1959. The importance of cardiac glycogen for the maintenance of life in foetal lambs and new-born animals during anoxia. Journal of Physiology (London) 146: 516-538.
- DAWSON, R.M.C.; ELLIOTT, D.C.; ELLIOTT, W.H.; JONES, K.M. 1969. Data for biochemical research, p. 507-508. Oxford: Clarendon Press.

- DE FRONZO, R.A.; TOBIN, J.D.; ANDRES, R. 1979. Glucose clamp technique: a method for quantifying insulin secretion and resistance. American Journal of Physiology 237: E214-E223.
- DHAND, U.K.; JEACOCK, M.K.; SHEPHERD, D.A.L.; SMITH, E.M.; VARNAM, G.C.E. 1970. Activities of enzymes concerned with pyruvate, oxaloacetate, citrate, acetate and acetoacetate metabolism in placental cotyledons of sheep. Biochimica et Biophysica Acta 222: 216-218.
- DOUMAS, B.T.; WATSON, W.A.; BIGGS, H.G. 1971. Albumin standards and the measurement of serum albumin with bromocresol green. Clinica Chimica Acta 31: 87-96,
- DUNN, A.; CHENOWETH, M.; BEVER, K. 1977. Use of ^3H and ^{14}C doubly labeled glucose and amino acids in the study of hormonal regulation of gluconeogenesis in rats. Federation Proceedings. Federation of American Societies for Experimental Biology 36: 245-252.
- DUNN, A.; CHENOWETH, M.; SCHAEFFER, L.D. 1967. Estimation of glucose turnover and the Cori cycle using glucose-6-t- ^{14}C . Biochemistry 6: 6-11.
- EDWARDS, E.M.; DHAND, U.K.; JEACOCK, M.K.; SHEPHERD, D.A.L. 1975. Activities of enzymes concerned with pyruvate and oxaloacetate metabolism in the heart and liver of developing sheep. Biochimica et Biophysica Acta 399: 217-227.
- EDWARDS, E.M.; RATTENBURY, J.M.; VARNAM, G.C.E.; DHAND, U.K.; JEACOCK, M.K.; SHEPHERD, D.A.L. 1977. Enzyme activities in the sheep placenta during the last three months of pregnancy. Biochimica et Biophysica Acta 497: 133-143.
- FALOONA, G.R.; UNGER, R.H. 1974. Glucagon, p.317-330. In: Jaffe, B.M.; Behrman, H.R. Methods of hormone radio-immunoassay. New York: Academic Press.
- FELIG, P.; POZEFSKY, T.; MARLISS, E.; CAHILL, C.F. 1970. Alanine: key role in gluconeogenesis. Science 167: 1003-1004.
- FELIG, P.; SHERWIN, R. 1976. Carbohydrate homeostasis, liver and diabetes, p.149-171. In: Popper, H.; Schaffner, F. Progress in liver diseases. Vol. V. New York: Grune and Stratton.

- FELL, B. F.; CAMPBELL, R. M.; MACKIE, W. S.; WEEKES, T. E. C. 1972. Changes associated with pregnancy and lactation in some extra-reproductive organs of the ewe. Journal of Agricultural Science (Cambridge) 79: 397-407.
- FERRE, P.; PEGORIER, J.; ASSAN, R.; MARLISS, E. B.; GIRARD, J. 1978. Influence of exogenous cortisol and triglyceride feeding on glucose homeostasis in the fasted newborn rat. Pediatric Research 12: 751-756.
- FERRIS, T. F.; HERDSON, P. B.; DUNNILL, M. S.; LEE, M. R. 1969. Toxemia of pregnancy in sheep: a clinical, physiological, and pathological study. Journal of Clinical Investigation 48: 1643-1655.
- FILSELL, O. H.; JARRETT, I. G.; TAYLOR, P. H.; KEECH, D. B. 1969. Effects of fasting, diabetes and glucocorticoids on gluconeogenic enzymes in the sheep. Biochimica et Biophysica Acta 184: 54-63.
- FORD, E. J. H. 1962. The effect of dietary restriction on some liver constituents of sheep during late pregnancy and early lactation. Journal of Agricultural Science (Cambridge) 59: 67-75.
- FORD, E. J. H. 1963a. Oxaloacetate levels in normal and ketotic pregnant sheep. Research in Veterinary Science 4: 408-412.
- FORD, E. J. H. 1963b. Glucose utilization in pregnant sheep. Biochemical Journal 88: 427-435.
- FORD, E. J. H. 1963c. Glucose utilization in pregnant sheep by a continuous infusion isotope dilution method. Nature 197: 614.
- FORD, E. J. H. 1965. Glucose utilization in spontaneous pregnancy toxemia of sheep. British Veterinary Journal 121: 139-144.
- FRASER, A. H. H.; GODDEN, W.; SNOOK, L. C.; THOMSON, W. 1939. Ketonaemia in pregnant ewes and its possible relation to pregnancy disease. Journal of Physiology (London) 97: 120-127.
- GALLAGHER, C. H. 1959. Biochemical studies on ovine pregnancy toxemia. I. Enzymic activities of liver and brain. Australian Journal of Agricultural Research 10: 854-864.
- GARFINKEL, D. 1969. Construction of biochemical computer models. Federation of European Biochemical Societies, Letters (Supplement) 2: S9-S13.

- GILL, J. C.; THOMSON, W. 1954. The production of ketonaemia and pregnancy toxaemia in ewes through manipulation of the feeding level. Journal of Agricultural Science (Cambridge) 45: 229-240.
- GRAHAM, N. McC. 1964. Energetic efficiency of fattening sheep. II. Effects of undernutrition. Australian Journal of Agricultural Research 15: 113-126.
- GRESHAM, E. L.; JAMES, E. J.; RAYE, J. R.; BATTAGLIA, F. C.; MAKOWSKI, E. L.; MESCHIA, G. 1972. Production and excretion of urea by the fetal lamb. Pediatrics 50: 372-379.
- GROENEWALD, J. W.; GRAF, H.; BEKKER, P. M.; MALAN, J. R.; CLARK, R. 1941a. Domsiekte or pregnancy disease in sheep - II. Onderstepoort Journal of Veterinary Science and Animal Industry 17: 245-296.
- GROENEWALD, J. W.; GRAF, H.; CLARK, R. 1941b. Domsiekte or pregnancy disease in sheep - I. A review of the literature. Onderstepoort Journal of Veterinary Science and Animal Industry 17: 225-244.
- GRUMBACH, M. M.; KAPLAN, S. L.; SCIARRA, J. J.; BURR, I. M. 1968. Chorionic growth hormone - prolactin (CGP): secretion, disposition, biologic activity in man, and postulated function as the "growth hormone" of the second half of pregnancy. New York Academy of Sciences, Annals 148: 501-531.
- HANDWERGER, S.; CRENSHAW, C. Jr.; MAURER, W. F.; BARRETT, J.; HURLEY, T. W.; GOLANDER, A.; FELLOWS, R. E. 1977. Studies on ovine placental lactogen secretion by homologous radioimmunoassay. Journal of Endocrinology 72: 27-34.
- HANDWERGER, S.; FELLOWS, R. E.; CRENSHAW, M. C.; HURLEY, T.; BARRETT, J.; MAURER, W. F. 1976. Ovine placental lactogen: acute effects on intermediary metabolism in pregnant and non-pregnant sheep. Journal of Endocrinology 69: 133-137.
- HAZZARD, T. C.; RUSSELL, A. M. 1968. Treatment of pregnancy toxaemia in sheep. Veterinary Record 82: 359-360.
- HEANEY, D. P.; LODGE, G. A. 1974. Energy metabolism during late pregnancy in the ewe, p.123-126. In: Menke, K. H.; Lantzsich, H.-J.; Reichl, J. R. Energy metabolism of farm animals. (European Association for Animal Production EAAP Publication 14) Universitat Hohenheim: Dokumentationsstelle.

- HEITMANN, R.N.; BERGMAN, E.N. 1978. Glutamine metabolism, interorgan transport, and glucogenicity in the sheep. American Journal of Physiology 234: E197-E203.
- HEITZMAN, R.J.; HERRIMAN, I.D.; AUSTIN, A.R. 1977. The response of sheep with pregnancy toxemia to trenbolone acetate. Veterinary Record 100: 317-318.
- HERRIMAN, I.D.; HEITZMAN, R.J. 1978. The effects of fasting on the concentrations of intermediate metabolites in the blood and hepatic tissues of pregnant and non-pregnant ewes. Journal of Agricultural Science (Cambridge) 90: 579-585.
- HEVESY, G.; JACOBSEN, C.F. 1940. Rate of passage of water through capillary and cell walls. Acta Physiologica Scandinavia 1: 11-18.
- HITCHCOCK, M.W.S. 1949. Fructose in the sheep foetus. Journal of Physiology (London) 108: 117-126.
- HO, P.; REBER, E.F. 1957. Effects of glucagon on hypoglycemia and ketonemia in pregnant ewes. American Journal of Veterinary Research 18: 342-344.
- HODGSON, J.C.; MELLOR, D.J. 1977. Maternal-foetal glucose kinetics in conscious, catheterised sheep. Proceedings of the Nutrition Society 36: 33-40.
- HOLM, L.W. 1958. Studies on the treatment of ovine pregnancy toxemia with corticosteroids and ACTH. Cornell Veterinarian 48: 348-357.
- HOPKINS, P.S. 1975. The development of the foetal ruminant, p. 1-14. In: McDonald, I.W.; Warner, A.C.I. Digestion and metabolism in the ruminant. (IV International Symposium on Ruminant Physiology). Armidale: University of New England.
- HOPKIRK, C.S.M. 1934. Pregnancy toxemia of ewes. Australian Veterinary Journal 10: 59-64.
- HORINO, M.; MACHLIN, L.J.; HERTELENDY, F.; KIPNIS, D.M. 1968. Effect of short-chain fatty acids on plasma insulin in ruminant and nonruminant species. Endocrinology 83: 118-128.
- HORSFIELD, S.; INFELD, J.M.; ANNISON, E.F. 1974. Compartmental analysis and model building in the study of glucose kinetics in the lactating cow. Proceedings of the Nutrition Society 33: 9-15.

- HORTON, E.S.; JOHNSON, C.; LEBOVITZ, H.E. 1968. Carbohydrate metabolism in uremia. Annals of Internal Medicine 68: 63-74.
- HOVE, K.; BLOM, A.K. 1976. Plasma insulin and growth hormone concentrations in pregnant sheep. I: Diurnal variations in mid- and late pregnancy. Acta Endocrinologica 82: 544-552.
- HOWARD, R.B.; PESCH, L.A. 1968. Respiratory activity of intact, isolated parenchymal cells from rat liver. Journal of Biological Chemistry 243: 3105-3109.
- HUNT, E.R. 1976. Treatment of pregnancy toxæmia in ewes by induction of parturition. Australian Veterinary Journal 52: 338-339.
- HUNTER, W.M.; GREENWOOD, F.C. 1962. Preparation of iodine-131 labelled human growth hormone of high specific activity. Nature 194: 495-496.
- HURST, R.O. 1967. A simplified approach to the use of determinants in the calculation of the rate equation for a complex enzyme system. Canadian Journal of Biochemistry 45: 2015-2039.
- INFIELD, J.M.; ROBB, J.; HORSFIELD, S.; ANNISON, E.F. 1974. Glucose biokinetics in lactating cows of defined energy status, p. 35-38. In: Menke, K.H.; Lantzsich, H-J.; Reichl, J.R. Energy metabolism of farm animals. (European Association for Animal Production EAAP Publication 14) Universität Hohenheim: Dokumentationsstelle.
- JAMES, E.; MESCHIA, G.; BATTAGLIA, F.C. 1971. A-V differences of free fatty acids and glycerol in the ovine umbilical circulation. Proceedings of the Society for Experimental Biology and Medicine 138: 823-826.
- JAMES, E.J.; RAYE, J.R.; GRESHAM, E.L.; MAKOWSKI, E.L.; MESCHIA, G.; BATTAGLIA, F.C. 1972. Fetal oxygen consumption, carbon dioxide production, and glucose uptake in a chronic sheep preparation. Pediatrics 50: 361-371.

- JARRETT, I. G.; FILSELL, O. H.; BALLARD, F. J. 1974. Metabolic and endocrine interrelationships in normal and diabetic sheep, p. 111-116. In: Levine, R.; Pfeiffer, E. F. Lipid metabolism, obesity and diabetes mellitus: impact upon atherosclerosis. (Hormone and Metabolic Research. Supplement Series No. 4) Stuttgart: Georg Thieme Publishers.
- JONES, C. T. 1976. Hypoglycaemia as a stimulus for adrenocorticotrophin secretion in foetal sheep. Journal of Endocrinology 70: 321-322.
- JORDAN, H. N.; PHILLIPS, R. W. 1978. Effect of fatty acids on isolated ovine pancreatic islets. American Journal of Physiology 234: E162-E167.
- JOST, J-P.; RICKENBERG, H. V. 1971. Cyclic AMP. Annual Review of Biochemistry 40: 741-774.
- JUDSON, G. J.; ANDERSON, E.; LUICK, J. R.; LENG, R. A. 1968. The contribution of propionate to glucose synthesis in sheep given diets of different grain content. British Journal of Nutrition 22: 69-75.
- JUDSON, G. J.; LENG, R. A. 1968. Effect of diet on glucose synthesis in sheep. Proceedings of the Australian Society of Animal Production 7: 354-358.
- JUDSON, G. J.; LENG, R. A. 1972. Estimation of the total entry rate and resynthesis of glucose in sheep using glucoses uniformly labelled with ^{14}C and variously labelled with ^3H . Australian Journal of Biological Sciences 25: 1313-1332.
- JUDSON, G. J.; LENG, R. A. 1973a. Studies on the control of gluconeogenesis in sheep: effect of glucose infusion. British Journal of Nutrition 29: 159-174.
- JUDSON, G. J.; LENG, R. A. 1973b. Studies on the control of gluconeogenesis in sheep: effect of propionate, casein and butyrate infusions. British Journal of Nutrition 29: 175-195.
- KALKHOFF, R. K.; RICHARDSON, B. L.; BECK, P. 1969. Relative effects of pregnancy, human placental lactogen and prednisolone on carbohydrate tolerance in normal and subclinical diabetic subjects. Diabetes 18: 153-163.
- KAMMULA, R. G. 1976. Metabolism of ketone bodies by ovine brain in vivo. American Journal of Physiology 231: 1490-1494.

- KATZ, M.L.; BERGMAN, E.N. 1966. Acid-base and electrolyte equilibrium in ovine pregnancy ketosis. American Journal of Veterinary Research 27: 1285-1292.
- KATZ, M.L.; BERGMAN, E.N. 1969a. A method for simultaneous cannulation of the major splanchnic blood vessels of the sheep. American Journal of Veterinary Research 30: 655-661.
- KATZ, M.L.; BERGMAN, E.N. 1969b. Hepatic and portal metabolism of glucose, free fatty acids, and ketone bodies in the sheep. American Journal of Physiology 216: 953-960.
- KATZ, J.; DUNN, A. 1967. Glucose-2-t as a tracer for glucose metabolism. Biochemistry 6: 1-5.
- KAUFMAN, C.F.; BERGMAN, E.N. 1971. Renal glucose, free fatty acid, and ketone body metabolism in the unanesthetized sheep. American Journal of Physiology 221: 967-972.
- KAUFMAN, C.F.; BERGMAN, E.N. 1974. Renal ketone body metabolism and gluconeogenesis in normal and hypoglycemic sheep. American Journal of Physiology 226: 827-832.
- KAUFMAN, C.F.; BERGMAN, E.N. 1978. Renal function studies in normal and toxemic pregnant sheep. Cornell Veterinarian 68: 124-137.
- KEECH, D.B.; UTTER, M.F. 1963. Pyruvate carboxylase. II. Properties. Journal of Biological Chemistry 238: 2609-2614.
- KELLY, P.A.; ROBERTSON, H.A.; FRIESEN, H.G. 1974. Temporal pattern of placental lactogen and progesterone secretion in sheep. Nature 248: 435-437.
- KIM, Y.J.; FELIG, P. 1971. Plasma chorionic somatomammotropin levels during starvation in mid-pregnancy. Journal of Clinical Endocrinology and Metabolism 32: 864-867.
- KRAML, M. 1966. A semi-automated determination of phospholipids. Clinica Chimica Acta 13: 442-448.
- KREBS, H.A.; CORNELL, N.W.; LUND, P.; HEMS, R. 1974. Isolated liver cells as experimental material, p. 726-750. In: Lindquist, F.; Tygstrup, N. Regulation of hepatic metabolism. (Proceedings of the Alfred Benzon Symposium VI, Copenhagen 1973) New York: Academic Press.
- KREBS, H.A.; HEMS, R.; WEIDEMANN, M.J.; SPEAKE, R.N. 1966. The fate of isotopic carbon in kidney cortex synthesizing glucose from lactate. Biochemical Journal 101: 242-249.

- KRISMAN, C.R. 1962. A method for the colorimetric estimation of glycogen with iodine. Analytical Biochemistry 4: 17-23.
- KRONFELD, D.S. 1970. Ketone body metabolism, its control, and its implications in pregnancy toxemia, acetonaemia, and feeding standards, p.566-583. In: Phillipson, A. T. Physiology of digestion and metabolism in the ruminant. Newcastle Upon Tyne: Oriel Press.
- KRONFELD, D.S. 1972. Ketosis in pregnant sheep and lactating cows. A review. Australian Veterinary Journal 48: 680-687.
- KRONFELD, D.S. 1977. Glucose transport and recycling determined by means of two tracers and multicompartmental analysis. Federation Proceedings. Federation of American Societies for Experimental Biology 36: 259-264.
- KRONFELD, D.S.; RAGGI, F. 1966. Decreases in concentrations of the nicotinamide coenzymes in the livers of fasting pregnant sheep and of field cases of pregnancy toxemia. Research in Veterinary Science 7: 499-503.
- KRONFELD, D.S.; RAMBERG, C.F.Jr.; SHAMES, D.M. 1971. Multicompartmental analysis of glucose kinetics in normal and hypoglycemic cows. American Journal of Physiology 220: 886-893.
- KRONFELD, D.S.; SIMESSEN, M.G. 1961. Glucose biokinetics in ovine pregnancy toxemia. Cornell Veterinarian 51: 478-488.
- KRONFELD, D.S.; SIMESSEN, M.G.; DUNGWORTH, D.L. 1960. Liver glycogen in normal and ketotic cows. Research in Veterinary Science 1: 242-247.
- LASKOV, R.; MARGOLIASH, E. 1963. Properties of high molecular weight glycogen from rat liver. Bulletin of the Research Council of Israel 11A4: 351-362.
- LEMONS, J.A.; ADCOCK, E.W.III; JONES, M.D.Jr.; NAUGHTON, M.A.; MESCHIA, G.; BATTAGLIA, F.C. 1976. Umbilical uptake of amino acids in the unstressed fetal lamb. Journal of Clinical Investigation 58: 1428-1434.
- LENG, R.A. 1965. Ketone body metabolism in normal and underfed pregnant sheep and in pregnancy toxemia. Research in Veterinary Science 6: 433-441.
- LENG, R.A. 1970. Glucose synthesis in ruminants. Advances in Veterinary Science 14: 209-260.

- LINDSAY, D.B.; LEAT, W.M.F. 1975. Carbohydrate and lipid metabolism, p.45-62. In: Blunt, M.H. The blood of sheep. Composition and function. New York: Springer-Verlag.
- LINDSAY, D.B.; SETCHELL, B.P. 1976. The oxidation of glucose, ketone bodies and acetate by the brain of normal and ketonaemic sheep. Journal of Physiology (London) 259: 801-823.
- LINDSAY, D.B.; STEEL, J.W.; BUTTERY, P.J. 1977. The net exchange of amino acids from muscle of fed and starved sheep. Proceedings of the Nutrition Society 36: 33A.
- LUNDBAEK, K. 1962. Intravenous glucose tolerance as a tool in definition and diagnosis of diabetes mellitus. British Medical Journal 1: 1507-1513.
- MACHLIN, L.J.; TAKAHASHI, Y.; HORINO, M.; HERTELENDY, F.; GORDON, R.S.; KIPNIS, D. 1968. Regulation of growth hormone secretion in non-primate species, p.292-305. In: Pecile, A.; Muller, E.E. Growth hormone. (Proceedings of the First International Symposium, Milan, 1967, International Congress Series, 158). Amsterdam: Excerpta Medica.
- MACKIE, W.S.; CAMPBELL, R.M. 1972. Effects of pregnancy and lactation on the activities of some gluconeogenic and urea-cycle enzymes in sheep liver. Journal of Agricultural Science (Cambridge) 79: 423-429.
- MARSH, W.H.; FINGERHUT, B.; MILLER, H. 1965. Automated and manual direct methods for the determination of blood urea. Clinical Chemistry 11: 624-627.
- MARTIN, R.J.; WILSON, L.L.; COWAN, R.L.; SINK, J.D. 1973. Effects of fasting and diet on enzyme profiles in ovine liver and adipose tissue. Journal of Animal Science 36: 101-106.
- MCCAUSLAND, I.P.; O'HARA, P.J.; HERDSON, P.B.; LEET, N.G. 1974. Spontaneous toxæmia of pregnancy in sheep. A study of renal function and glomerular fine structure. Journal of Comparative Pathology 84: 375-380.
- McCLYMONT, G.L.; SETCHELL, B.P. 1955a. Ovine pregnancy toxæmia. I. Tentative identification as a hypoglycaemic encephalopathy. Australian Veterinary Journal 31: 53-68.
- McCLYMONT, G.L.; SETCHELL, B.P. 1955b. Ovine pregnancy toxæmia. II. Experimental therapy with glycerol and glucose. Australian Veterinary Journal 31: 170-174.

- McCLYMONT, G.L.; SETCHELL, B.P. 1956a. Ovine pregnancy toxæmia. III. Further evidence of the correlation of depth of hypoglycaemia and induction of symptoms. Australian Veterinary Journal 32: 22-25.
- McCLYMONT, G.L.; SETCHELL, B.P. 1956b. Ovine pregnancy toxæmia. IV. Insulin induced hypoglycaemic encephalopathy in the sheep and its implications as regards pathogenesis of the disease. Australian Veterinary Journal 32: 97-109.
- McINTOSH, G.H.; FILSELL, O.H.; JARRETT, I.G. 1973. Kidney function and net glucose production in normal and acidotic sheep. Australian Journal of Biological Sciences 26: 1389-1394.
- MELLOR, D.J.; MATHESON, I.C. 1975. Chronic catheterisation of the aorta and umbilical vessels of fetal sheep. Research in Veterinary Science 18: 221-223.
- MELLOR, D.J.; MATHESON, I.C.; SMALL, J. 1977a. Some changes in the composition of maternal and fetal plasma from chronically catheterised sheep during short periods of reduced feed intake in late pregnancy. Research in Veterinary Science 23: 119-121.
- MELLOR, D.J.; MATHESON, I.C.; SMALL, J. 1977b. Changes in the corticosteroid concentrations of plasma from single and twin fetuses during the last 3 weeks of pregnancy in sheep. Journal of Reproduction and Fertility 50: 383-385.
- MIRSKI, A.; ROSENBAUM, I.; STEIN, L.; WERTHEIMER, E. 1938. On the behaviour of glycogen after diets rich in protein and in carbohydrate. Journal of Physiology (London) 92: 48-61.
- MORTON, J.L.; BUTTERY, P.J.; LINDSAY, D.B. 1977. Gluconeogenesis from amino acids by isolated sheep liver cells. Proceedings of the Nutrition Society 36: 20A.
- O'HARA, P.J.; WOLFF, J.E.; OLIVER, R.E. 1975. The effect of starvation on the pregnant ewe. Proceedings of the New Zealand Veterinary Association Sheep Society (New Zealand Sheep Society 5th Seminar) p. 22-31.
- OLEFSKY, J.M.; FARQUHAR, J.W.; REAVEN, G.M. 1973. Do the oral and intravenous glucose tolerance tests provide similar diagnostic information in patients with chemical diabetes mellitus? Diabetes 22: 202-209.

- OSBALDISTON, G. W. 1971. The kidney: its function and evaluation in health and disease, p. 1-60. In: Kaneko, J. J.; Cornelius, C. E. Clinical biochemistry of domestic animals. Vol. II. 2nd ed. New York: Academic Press.
- PARRY, H. B. 1950. Toxaemias of pregnancy in the domestic animals with particular reference to the sheep, p. 85-93. In: Hammond, J.; Browne, F. J.; Wolstenholme, G. E. W. Toxaemias of pregnancy. (A Ciba Foundation Symposium) London: Churchill.
- PARRY, H. B.; TAYLOR, W. H. 1956. Renal function in sheep during normal and toxaemic pregnancies. Journal of Physiology (London) 131: 383-392.
- PATTERSON, D. S. P.; BURNS, K. N.; CUNNINGHAM, N. F.; HEBERT, C. N.; SABA, N. 1964. Plasma concentrations of glucose and non-esterified fatty acids (NEFA) in the pregnant and lactating ewe and the effect of dietary restriction. Journal of Agricultural Science (Cambridge) 62: 253-262.
- PAXSON, C. L. Jr; MORRISS, F. H. Jr; ADCOCK, E. W. III. 1978. Effect of uterine artery insulin infusions on umbilical glucose uptake in sheep. Pediatric Research 12: 864-867.
- PEARCE, J.; UNSWORTH, E. F. 1976. The effects of grass and concentrate diets on the specific activities of some enzymes of hepatic carbohydrate metabolism in sheep. British Journal of Nutrition 35: 407-411.
- PHILIPPS, A. F.; CARSON, B. S.; MESCHIA, G.; BATTAGLIA, F. C. 1978. Insulin secretion in fetal and newborn sheep. American Journal of Physiology 235: E467-E474.
- PITTS, P. F. 1968. Physiology of the kidney and body fluids. 2nd ed. Chicago: Year Book Medical Publishers.
- POGSON, C. I.; LONGSHAW, I. D.; ROOBOL, A.; SMITH, S. A.; ALLEYNE, G. A. O. 1976. Phosphoenolpyruvate carboxykinase and renal gluconeogenesis, p. 335-368. In: Hanson, R. W.; Mehlman, M. A. Gluconeogenesis: its regulation in mammalian species. New York: Wiley-Interscience.
- PRIOR, R. L.; CHRISTENSON, R. K. 1977. Gluconeogenesis from alanine in vivo by the ovine fetus and lamb. American Journal of Physiology 233: E462-E468.
- PRIOR, R. L.; CHRISTENSON, R. K. 1978a. Insulin and glucose effects on glucose metabolism in pregnant and nonpregnant ewes. Journal of Animal Science 46: 201-210.

- PRIOR, R.L.; CHRISTENSON, R.K. 1978b. Insulin and glucose effects on amino acid metabolism in pregnant and nonpregnant ewes. Journal of Animal Science 46: 211-218.
- PROCOS, J. 1962a. Ovine ketosis. II. The effect of pregnancy on the blood ketone body levels of well-fed ewes. Onderstepoort Journal of Veterinary Research 29: 107-110.
- PROCOS, J. 1962b. Ovine ketosis. III. - The effect of starvation on the blood sugar and ketone body levels of wethers. Onderstepoort Journal of Veterinary Research 29: 259-267.
- PROCOS, J.; GILCHRIST, F.M.C. 1966. Ovine ketosis. V. Ketone body and blood glucose levels of merino ewes during the pre-clinical, clinical and postclinical stages of pregnancy disease. Onderstepoort Journal of Veterinary Research 33: 161-167.
- PUGH, P.S. 1954. Hyperketonaemia and foetal death in the sheep. Veterinary Record 66: 645-652.
- RADZIUK, J.; NORWICH, K.H.; VRANIC, M. 1978. Experimental validation of measurements of glucose turnover in nonsteady state. American Journal of Physiology 234: E84-E93.
- RANDALL, G.C.B. 1979. Studies on the effect of acute asphyxia on the fetal pig in utero. Biology of the Neonate 36: 63-69.
- REID, R.L. 1951a. Studies on the carbohydrate metabolism of sheep. III. The blood glucose during insulin hypoglycaemia. Australian Journal of Agricultural Research 2: 132-145.
- REID, R.L. 1951b. Studies on the carbohydrate metabolism of sheep. IV. Hypoglycaemic signs and their relationship to blood glucose. Australian Journal of Agricultural Research 2: 146-157.
- REID, R.L. 1958. Studies on the carbohydrate metabolism of sheep. VII. Intravenous glucose and acetate tolerance tests. Australian Journal of Agricultural Research 9: 788-796.
- REID, R.L. 1960a. Studies on the carbohydrate metabolism of sheep. IX. Metabolic effects of glucose and glycerol in undernourished pregnant ewes and in ewes with pregnancy toxemia. Australian Journal of Agricultural Research 11: 42-57.
- REID, R.L. 1960b. Studies on the carbohydrate metabolism of sheep. X. Further studies on hypoglycaemia and hyperketonaemia in undernourished pregnant ewes and in ewes with pregnancy toxemia. Australian Journal of Agricultural Research 11: 346-363.

- REID, R.L. 1960c. Studies on the carbohydrate metabolism of sheep. XI. The role of the adrenals in ovine pregnancy toxæmia. Australian Journal of Agricultural Research 11: 364-382.
- REID, R.L. 1960d. Studies on the carbohydrate metabolism of sheep. XII. Further studies on the diabetic nature of the metabolic abnormalities in ovine pregnancy toxæmia. Australian Journal of Agricultural Research 11: 530-538.
- REID, R.L. 1962. Studies on the carbohydrate metabolism of sheep. XV. The adrenal response to the climatic stresses of cold, wind and rain. Australian Journal of Agricultural Research 13: 296-306.
- REID, R.L. 1968. The physiopathology of undernourishment in pregnant sheep, with particular reference to pregnancy toxæmia. Advances in Veterinary Science 12: 163-238.
- REID, R.L.; HOGAN, J.P. 1959. Studies on the carbohydrate metabolism of sheep. VIII. Hypoglycaemia and hyperketonaemia in undernourished and fasted pregnant ewes. Australian Journal of Agricultural Research, 10: 81-96.
- REID, R.L.; MILLS, S.C. 1962. Studies on the carbohydrate metabolism of sheep. XIV. The adrenal response to psychological stress. Australian Journal of Agricultural Research, 13: 282-295.
- REILLY, P.E.B.; CHANDRASENA, L.G. 1978. Glucose lactate interrelations in sheep. American Journal of Physiology 235: E487-E492.
- REILLY, P.E.B.; FORD, E.J.H. 1971. The effects of different dietary contents of protein on amino acid and glucose production and on the contribution of amino acids to gluconeogenesis in sheep. British Journal of Nutrition 26: 249-263.
- RICHARDSON, R.I.; LIVESEY, A.F. 1979. Gluconeogenesis in isolated sheep hepatocytes. Proceedings of the Nutrition Society 38(1): 2A.
- ROBINSON, J.J. 1977. The influence of maternal nutrition on ovine foetal growth. Proceedings of the Nutrition Society 36: 9-16.
- RODERICK, L.M.; HARSHFIELD, G.S.; MERCHANT, W.R. 1933. Further observations on the functional pathology of pregnancy disease of ewes. Cornell Veterinarian 23: 348-353.

- ROSS, J. P.; KITTS, W. D. 1973. Relationship between post-, prandial plasma volatile fatty acids, glucose and insulin levels in sheep fed different feeds. Journal of Nutrition 103: 488-493.
- SABA, N.; BURNS, K. N.; CUNNINGHAM, N. F.; HEBERT, C. N.; PATTERSON, D. S. P. 1966. Some biochemical and hormonal aspects of experimental ovine pregnancy toxemia. Journal of Agricultural Science (Cambridge) 67: 129-138.
- SABA, N.; CUNNINGHAM, N. F. 1971. Plasma corticosteroid levels in ovine pregnancy toxemia and hypocalcaemia. Research in Veterinary Science 12: 483-485.
- SACCA, L.; CRYER, P. E.; SHERWIN, R. S. 1979. Blood glucose regulates the effects of insulin and counterregulatory hormones on glucose production in vivo. Diabetes 28: 533-536.
- SALWAY, J. G. 1969. The simultaneous determination of acetoacetate and glucose in capillary blood. Clinica Chimica Acta 25: 109-116.
- SASAKI, S.; AMBO, K.; MURAMATSU, M.; TSUDA, T. 1975. Gluconeogenesis in the kidney-cortex slices of normal fed and starved sheep. Tohoku Journal of Agricultural Research 26: 20-29.
- SCARISBRICK, R.; PUGH, P. D. S. 1957. The passage of acetate across the placenta and its uptake by the foetal sheep. British Veterinary Journal 113: 328-341.
- SCRUTTON, M. C.; UTTER, M. F. 1968. The regulation of glycolysis and gluconeogenesis in animal tissues. Annual Review of Biochemistry 37: 249-302.
- SEAMAN, I. 1855. On parturient fever in ewes, "Giddiness accompanying parturition". Journal of the Royal Agricultural Society of England 15: 383-389.
- SEGLIN, P. O. 1976. Preparation of isolated rat liver cells, p. 29-83. In: Prescott, D. M. Methods in cell biology. Vol. XIII, New York: Academic Press.
- SETCHELL, B. P.; BASSETT, J. M.; HINKS, N. T.; GRAHAM, N. McC. 1972. The importance of glucose in the oxidative metabolism of the pregnant uterus and its contents in conscious sheep with some preliminary observations on the oxidation of fructose and glucose by fetal sheep. Quarterly Journal of Experimental Physiology 57: 257-266.

- SETCHELL, B. P.; WAITES, G. M. H. 1962. Adrenaline release during insulin hypoglycaemia in the sheep. Journal of Physiology (London) 164: 200-209.
- SHELLEY, H. J. 1961. Glycogen reserves and their changes at birth and in anoxia. British Medical Bulletin 17: 137-143.
- SHERWIN, R. S.; KRAMER, K. J.; TOBIN, J. D.; INSEL, P. A.; LILJENQUIST, J. E.; BERMAN, M.; ANDRES, R. 1974. A model of the kinetics of insulin in man. Journal of Clinical Investigation 53: 1481-1492.
- SHIPLEY, R. A.; CLARK, R. E. 1972. Tracer methods for in vivo kinetics: theory and applications. New York: Academic Press
- SILVERSTONE, F. A.; SOLOMONS, E.; RUBRICUS, J. 1961. The rapid intravenous glucose tolerance test in pregnancy. Journal of Clinical Investigation 40: 2180-2189.
- SIMMONS, M. A.; BURD, L. I.; LEMONS, J. A.; JONES, M. D. Jr.; SCHREINER, R. L.; MESCHIA, G.; BATTAGLIA, F. C. 1975. Placental utilization and conversion of glucose in vivo. Pediatric Research 9: 279.
- SIMMONS, M. A.; JONES, M. D. Jr.; BATTAGLIA, F. C.; MESCHIA, G. 1978. Insulin effect on fetal glucose utilization. Pediatric Research 12: 90-92.
- SIMMONS, M. A.; MESCHIA, G.; MAKOWSKI, E. L.; BATTAGLIA, F. C. 1974. Fetal metabolic response to maternal starvation. Pediatric Research 8: 830-836.
- SKEGGS, L. T.; HOCHSTRASSER, H. 1964. Multiple automatic sequential analysis. Clinical Chemistry 10: 918-936.
- SLATER, J. S.; MELLOR, D. J. 1979. Concentrations of free amino acids in maternal and fetal plasma from conscious catheterised ewes during the last five weeks of pregnancy. Research in Veterinary Science 26: 296-301.
- SMITH, E. E.; GOETSCH, G. D.; JACKSON, H. D. 1961. Oxidation of volatile fatty acids by rumen epithelium and by liver from ketotic sheep. Archives of Biochemistry and Biophysics 95: 256-262.
- SNOOK, L. C. 1939. Fatty infiltration of the liver in pregnant ewes. Journal of Physiology (London) 97: 238-249.

- SOMOGYI, M. 1945. Determination of blood sugar. Journal of Biological Chemistry 160: 69-73.
- SPELLACY, W.N.; GOETZ, F.C. 1963. Plasma insulin in normal late pregnancy. New England Journal of Medicine 268: 988-991.
- STEEL, J.W.; LENG, R.A. 1968. Effect of plane of nutrition and pregnancy on glucose entry rates in sheep. Proceedings of the Australian Society of Animal Production 7: 342-347.
- STEEL, J.W.; LENG, R.A. 1973a. Effects of plane of nutrition and pregnancy on gluconeogenesis in sheep. 1. The kinetics of glucose metabolism. British Journal of Nutrition 30: 451-473.
- STEEL, J.W.; LENG, R.A. 1973b. Effects of plane of nutrition and pregnancy on gluconeogenesis in sheep. 2. Synthesis of glucose from ruminal propionate. British Journal of Nutrition 30: 475-489.
- STERN, J.S.; BAILE, C.A.; MAYER, J. 1970. Are propionate and butyrate physiological regulators of plasma insulin in ruminants? American Journal of Physiology 219: 84-91.
- STEVENSON, R.E.; MORRISS, F.H.; ADCOCK, E.W.III; HOWELL, R.R. 1976. Development of gluconeogenic enzymes in fetal sheep liver and kidney. Developmental Biology 52: 167-172.
- SUTTON, J.D.; NICHOLSON, J.W.G. 1968. The digestion of energy and starch along the gastro-intestinal tract of sheep. Proceedings of the Nutrition Society 27: 49A-50A.
- SWENSON, M.J. 1970. Physiologic properties, cellular and chemical constituents of blood, p. 52. In: Swenson, M.J. Duke's physiology of domestic animals. 8th ed. Ithaca: Cornell University Press.
- SYKES, A.R.; FIELD, A.C. 1972. Effects of dietary deficiencies of energy, protein and calcium on the pregnant ewe. III. Some observations on the use of biochemical parameters in controlling energy undernutrition during pregnancy and on the efficiency of utilization of energy and protein for foetal growth. Journal of Agricultural Science (Cambridge) 78: 127-133.
- SYKES, A.R.; FIELD, A.C. 1973. Effects of dietary deficiencies of energy, protein and calcium on the pregnant ewe. IV. Serum total protein, albumin, globulin, transferrin and plasma urea levels. Journal of Agricultural Science (Cambridge) 80: 29-36.

- SYKES, A.R.; FIELD, A.C. 1974. Seasonal changes in plasma concentrations of proteins, urea, glucose, calcium and phosphorus in sheep grazing a hill pasture and their relationship to changes in body composition. Journal of Agricultural Science (Cambridge) 83: 161-169.
- SYKES, A.R.; THOMPSON, R. 1978. The relationship between serum albumin concentration and body protein loss in pregnant sheep. Journal of Agricultural Science (Cambridge) 91: 173-179.
- TASKER, J.B. 1971. Fluids, electrolytes, and acid-base balance, p.61-110. In: Kaneko, J.J.; Cornelius, C.E. Clinical biochemistry of domestic animals. Vol. II. 2nd ed. New York: Academic Press.
- TAYLOR, M.J.; JENKIN, G.; CHAN, J.S.D.; FRIESEN, H.G.; THORBURN, G.D.; ROBINSON, J.S. 1978. Ovine placental lactogen in maternal and foetal plasma in sheep. Journal of Endocrinology 79: 39P-40P.
- TAYLOR, P.H.; WALLACE, J.C.; KEECH, D.B. 1971. Glucogenic enzymes in sheep liver. Intracellular localization of pyruvate carboxylase and phosphoenolpyruvate carboxykinase in normal, fasted and diabetic sheep. Biochimica et Biophysica Acta 237: 179-191.
- THOMPSON, J.R.; CHRISTOPHERSON, R.J.; HAMMOND, V.A.; HILLS, G.A. 1978. Effects of acute cold exposure on plasma concentrations of noradrenaline and adrenaline in sheep. Canadian Journal of Animal Science 58: 23-28.
- THOMSON, A.M.; THOMSON, W. 1949. Lambing in relation to the diet of the pregnant ewe. British Journal of Nutrition 2: 290-305.
- THOMSON, G.G. 1956. Observations on some treatments of ovine pregnancy toxæmia. New Zealand Veterinary Journal 4: 136-144.
- TRENKLE, A. 1972. Radioimmunoassay of plasma hormones: review of plasma insulin in ruminants. Journal of Dairy Science 55: 1200-1211.
- TRENKLE, A. 1976. Estimates of the kinetic parameters of growth hormone metabolism in fed and fasted calves and sheep. Journal of Animal Science 43: 1035-1043.
- TRINDER, P. 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Annals of Clinical Biochemistry 6: 24-27.

- TSOULOS, N.G.; COLWILL, J.R.; BATTAGLIA, F.C.; MAKOWSKI, E.L.; MESCHIA, G. 1971. Comparison of glucose, fructose, and O₂ uptakes by fetuses of fed and starved ewes. American Journal of Physiology 221: 234-237.
- UNDERWOOD, E.J.; CURNOW, D.H.; SHIER, F.L. 1943. Further biochemical studies of pregnancy toxemia in sheep. Australian Veterinary Journal 19: 164-173.
- UNGER, R.H. 1971. Glucagon and the insulin:glucagon ratio in diabetes and other catabolic illnesses. Diabetes 20: 834-838.
- VAN RENSBURG, S. 1931. Pregnancy disease in ewes. A -In South Africa. Veterinary Record 46: 1147-1148.
- VARNAM, G.C.E.; JEACOCK, M.K.; SHEPHERD, D.A.L. 1978. Activities of ketone body utilising enzymes in tissues of fed and fasted sheep. Research in Veterinary Science 24: 124-125.
- WAGLE, S.R.; NELSON, P. 1966. Studies on activities of gluconeogenic enzymes in sheep liver. Biochimica et Biophysica Acta 121: 190-191.
- WALLACE, L.R. 1948. The growth of lambs before and after birth in relation to the level of nutrition. Journal of Agricultural Science (Cambridge) 38: 367-401.
- WARNES, D.M.; SEAMARK, R.F.; BALLARD, F.J. 1977a. Metabolism of glucose, fructose and lactate in vivo in chronically cannulated foetuses and in suckling lambs. Biochemical Journal 162: 617-626.
- WARNES, D.M.; SEAMARK, R.F.; BALLARD, F.J. 1977b. The appearance of gluconeogenesis at birth in sheep. Activation of the pathway associated with blood oxygenation. Biochemical Journal 162: 627-634.
- WEBSTER, A.J.F.; HEITMAN, J.H.; HAYS, F.L.; OLYNYK, G.P. 1969. Catecholamines and cold thermogenesis in sheep. Canadian Journal of Physiology and Pharmacology 47: 719-724.
- WEEKES, T.E.C.; RICHARDSON, R.I.; GEDDES, N. 1979. The effect of ammonia on gluconeogenesis by isolated sheep liver cells. Proceedings of the Nutrition Society 38(1): 3A.

- WEST, C.E.; PASSEY, R.F. 1967. Effect of glucose load and of insulin on the metabolism of glucose and of palmitate in sheep. Biochemical Journal 102: 58-64.
- WHITE, R.G.; STEEL, J.W.; LENG, R.A.; LUICK, J.R. 1969. Evaluation of three isotope-dilution techniques for studying the kinetics of glucose metabolism in sheep. Biochemical Journal 114: 203-214.
- WOLFE, R.R.; ALLSOP, J.R.; BURKE, J.F. 1978. Fallibility of the intravenous glucose tolerance test as a measure of endogenous glucose turnover. Metabolism: Clinical and Experimental 27: 217-226.
- WOLFF, J.E. 1977. Loss of renal function in starved pregnant sheep. In: Agricultural research in the New Zealand Ministry of Agriculture and Fisheries 1976/77. (Annual Report of the Research Division) Hamilton: Government Printer.
- WOLFF, J.E.; BERGMAN, E.N. 1972. Gluconeogenesis from plasma amino acids in fed sheep. American Journal of Physiology 223: 455-460.
- WOLFF, J.E.; O'HARA, P.J.; KRONFELD, D.S. 1974. Effect of starvation on blood metabolites in pregnant sheep. Proceedings of the New Zealand Society of Animal Production 34: 66.
- YANG, Y.T.; BALDWIN, R.L. 1973a. Preparation and metabolism of isolated cells from bovine adipose tissue. Journal of Dairy Science 56: 350-365.
- YANG, Y.T.; BALDWIN, R.L. 1973b. Lipolysis in isolated cow adipose cells. Journal of Dairy Science 56: 366-374.
- YOUNG, J.W. 1977. Gluconeogenesis in cattle: significance and methodology. Journal of Dairy Science 60: 1-15.
- YOUNG, M.; McFADYEN, I.R. 1973. Placental transfer and fetal uptake of amino acids in the pregnant ewe. Journal of Perinatal Medicine 1: 174-182.
- ZIVIN, J.A.; SNARR, J.F. 1973. An automated colorimetric method for the measurement of 3-hydroxybutyrate concentration. Analytical Biochemistry 52: 456-461.

APPENDIX 1: Program WINN.INSULIN.

This program calculates extravascular insulin concentration from plasma insulin. It was programed in Basic by Arcus, A. C. and McKinnon, A., Clinical School, Christchurch, and adapted to run on the MAF ICL 2903 computer by M. Winn, Biometrics Section, Ruakura ARC; Hamilton.

A listing of the program and a copy of the output are shown in Table 32. On the interactive system, the program asks for the experiment number, and then the number of observations. The observation times, in minutes are entered, separated by commas, and the program then asks for the corresponding plasma insulin values (in $\mu\text{U/ml}$), which are also entered as a string separated by commas.

The program then lists against the times of observation, the calculated extravascular insulin concentrations. It then asks whether there is more data at the same times. If there is, the new insulin values are entered. If not, the next experiment number is entered, followed by the new observation times and insulin concentrations.

Equations:

a) Kinetically;

$$C_3(t_i) = C_3(0)e^{\frac{-k}{V_3} \cdot t_i} + \frac{k}{V_3} e^{\frac{-k}{V_3} \cdot t_i} \int_0^{t_i} C_1(T) e^{\frac{k}{V_3} \cdot T} dT$$

b) Program;

$$S = \sum \left(I(K) e^{0.02T(K)} + I(K-1) e^{0.02T(K-1)} \right) \left(\frac{T(K) - T(K-1)}{2} \right)$$

$$E(K) = I(1) e^{-0.02T(K)} + 0.02 e^{-0.02T(K)} \cdot S$$

In the program the integral (area under the curve) is calculated by the trapezoid rule, as S, and:

$$\frac{k}{V_3} = L = 0.02 \text{ (the value of } L_{13} \text{ calculated for the model of Sherwin et al., 1974).}$$

$I(K) = C_1(T) =$ plasma insulin concentration

$t_i = T(K) =$ observed time

$C_3(T) = E(K) =$ extravascular insulin concentration

$C_3(0) = I(1) =$ initial concentration in the extravascular compartment, which equals the initial plasma insulin concentration.

Derivation of the kinetic formula:

For a model with three compartments, compartment 1, plasma insulin, and compartment 3, extravascular insulin (from Sherwin et al., 1974), and

$M_3 =$ mass of insulin in compartment 3

K_{13} and $K_{31} =$ flow rate between the compartments, ml/min

C_1 and $C_3 =$ concentration of insulin, $\mu\text{U/ml}$.

then:

$$\frac{dM_3}{dt} = K_{31} C_1 - K_{13} C_3$$

$$M_3 = C_3 V_3$$

$$\frac{dM_3}{dt} = \frac{dC_3 \cdot V_3}{dt} + C_3 \cdot \frac{dV_3}{dt}$$

But $\frac{dV_3}{dt} = 0$

Therefore $V_3 \cdot \frac{dC_3}{dt} = K_{31} C_1 - K_{13} C_3$

$$\frac{dC_3}{dt} = \frac{K_{31}}{V_3} \cdot C_1 - \frac{K_{13}}{V_3} \cdot C_3 \quad (1)$$

An impulse response in compartment 3:

$$h_3(t) = e^{-\frac{K_{13} \cdot t}{V_3}}$$

and the input, R:

$$R_{31}(t) = \frac{K_{31}}{V_3} \cdot C_1(t)$$

And the general solution of equation 1:

$$C_3(t) = C_3(0) \cdot e^{-\frac{K_{13}}{V_3} \cdot t} + \frac{K_{31}}{V_3} \cdot e^{-\frac{K_{13}}{V_3} \cdot t} \int_0^t C_1(T) \cdot e^{\frac{K_{13}}{V_3} \cdot T} \cdot dT$$

TABLE 32: WINN. INSULIN: Calculation of Extravascular Insulin.

a) Program Listing

```

: LIST
10 REM PROGRAMME TO CALCULATE EXTRAVASCULAR INSULIN.
20 REM ACCORDING TO FORMULA ON PAGE 6
30 DIM I(50),E(50),T(50)
35 PRINT "EXPT NUMBER"
36 INPUT M
40 PRINT"ENTER THE NUMBER OF MEASUREMENTS",
50 INPUT N
80 MAT T = ZER(N)
90 PRINT"ENTER THE TIMES OF MEASUREMENT"
100 MAT INPUT T
101 MAT I= ZER(N)
102 MAT E = ZER(N)
110 PRINT"ENTER THE INSULIN VALUES"
120 MAT INPUT I
130 L=0.02
140 S=0
150 E(1)=I(1)*EXP(-L*T(1))
160 I1=I(1)
170 T1 = T(1)
180 FOR K=2 TO N
BREAKIN - 'C', 'I' OR 'S': C
190 T2=T(K)
200 I2 = I(K)
210 IF I2=0 GO TO 260
220 S = S + (I2*EXP(L*T2)+I1*EXP(L*T1))*(T2-T1)/2
230 E(K) = I(1)*EXP(-L*T2) + L*EXP(-L*T2)*S
240 I1 = I2
250 T1 = T2
260 NEXT K
270 PRINT
280 PRINT"THE TIMES AND EXTRAVASCULAR INSULIN VALUES ARE : "
281 FOR I=1 TO N
282 PRINT T(I),E(I)
283 NEXT I
300 PRINT
310 PRINT "IS THERE MORE DATA AT THE SAME TIMES,Y OR N",
320 INPUT QS
330 IF QS = "N" GOTO 350
340 GOTO 101
350 STOP
360 END

```

TABLE 32 (Continued)

b) WINN.INSULIN Output.

4			
4	* WINN.INSULIN	20 APR 79	17:13:43
4	EXPT NUMBER		
4	? 12		
4	ENTER THE NUMBER OF MEASUREMENTS		? 16
4	ENTER THE TIMES OF MEASUREMENT		
4	? 0,3.15,4,5,7.05,10.17,15.22,20,25,30,38.3,48,54.3,60.3,90,120		
4	ENTER THE INSULIN VALUES		
4	? 10.4,24.5,28,30,33.5,45,39,34,32.5,33,29.5,50,52,44,38,47		
4			
4	THE TIMES AND EXTRAVASCULAR INSULIN VALUES ARE :		
4	0	10.4	
4	3.15	10.8444	
4	4	11.1043	
4	5	11.4589	
4	7.05	12.2756	
4	10.17	13.919	
4	15.22	16.6055	
4	20	18.411	
4	25	19.8222	
4	30	21.0562	
4	38.3	22.6041	
4	48	25.825	
4	54.3	28.8208	
4	60.3	30.9689	
4	90	35.5994	
4	120	39.8938	
4			
4	IS THERE MORE DATA AT THE SAME TIMES,Y OR N ? N		
4	* .385 SECONDS USED	1/116:45	
4			
4	: BYE		
4	* FINISHED	17:17:18	
1	2	LGT(C)	

APPENDIX 2: Program GLUCOESA78

This program calculates the specific activity of glucose in plasma and was written in Fortran by M. E. Wastney. It calculates the specific activity of [^{14}C]- and [^3H] glucose when isolated as the potassium gluconate derivative. The activity is expressed as fraction of dose/mg glucose, for single injection experiments or as dpm/mg glucose for continuous infusions. A flow diagram of the program is presented in Fig. 23 and a program listing in Table 33a.

For each experiment two types of data card are required:

Card 1: (One for each experiment)

Column	Format	Variable name	Description
1 - 5	I5	N	Number of samples
6 - 10	I5	EXPT	Experiment number
11 - 15	F5.1	BKA	Background counts, channel A
16 - 20	F5.1	BKB	Background counts, channel B
21 - 32	F12.0	CDOSE	^{14}C counts injected
33 - 44	F12.0	TDOSE	^3H counts injected

(CDOSE and TDOSE = 1 for continuous infusion experiments)

Card 2: (one card/sample)

Column	Format	Variable name	Description
1 - 10	F10.1	F(I)	Sample time, min
11 - 20	F10.1	TCA	Counts in channel A, cpm
21 - 30	F10.1	CB	Counts in channel B, cpm
31 - 40	F10.1	G	Glucose in filtrate, mg/dl
41 - 50	F10.1	M	Mass of K-gluconate counted, mg
51 - 55	F5.1	V	Volume of filtrate analysed, ml
56 - 61	F6.4	S	External standard ratio
62 - 67	F6.2	PG	Plasma glucose, mg/dl

Theory and Calculations:

When samples containing ^3H and ^{14}C tracers are counted, the amount in each channel must be corrected for spillover of counts between channels. Channel A is mostly ^3H but also contains some ^{14}C and the fraction varies with external standard ratio (ESR). Channel B contains mainly ^{14}C .

To determine the ratio of ^{14}C counts in the ^3H channel (A), a ^{14}C sample was counted in channels A and B, with different amounts of quenching. The ratio of ^{14}C in channel A / ^{14}C counts in channel B was calculated, and the ratio plotted against each ESR value.

This function was incorporated into the program:

$$X = f(S)$$

$$\text{where } X = \frac{^{14}\text{C counts in channel A}}{^{14}\text{C counts in channel B}}$$

$$S = \text{ESR}$$

Knowing the counts in channel B, and the ESR, the ^{14}C in channel A:

$$^{14}\text{C in channel A} = \text{counts in B} \cdot X$$

$$CA = CB \cdot X$$

(In the program C refers to ^{14}C , and T refers to ^3H)

and the ^3H counts in channel A = Total counts in A - ^{14}C in A

$$TA = TCA - CA$$

Quenching curves of efficiency of counting versus ESR, were plotted for ^3H in channel A and for ^{14}C in channel B, and the parameters of these curves are used in the program to correct for counting efficiency, and convert cpm to dpm.

Specific Activity is calculated as dpm/mg glucose in two parts:

a) Determining mg glucose in original sample:

Weight of glucose in sample = mg in filtrate/ml . ml filtrate taken

$$GF = (G/100) \cdot V$$

b) Determining dpm:

The theoretical recovery of gluconate is calculated from the conversion factor:

$$M. W. \text{ gluconate} / M. W. \text{ glucose} = 1.30034$$

and the quantity of glucose present, which;

$$= \text{carrier glucose} + \text{glucose in sample}$$

$$= 100 + (G/100) \cdot V$$

$$= 100 + GF$$

Quantity of gluconate formed, Q, if all the glucose converted was recovered;

$$Q = 1.30034 \cdot (100 + GF)$$

Dpm with 100% recovery, if actual recovery is M;

$$\text{Total dpm} = (\text{dpm}/M) \cdot Q$$

Then, specific activity, SA;

$$SA = \text{Total dpm} / GF$$

Specific activity, as fraction of dose;

$$PSA = SA / \text{dose injected.}$$

Output: (Table 33b)

The output consists of two tables for each experiment. The first prints out all the input data in a convenient form, and in addition the ^3H counts in channel A and the efficiency of counting for each sample. The second table contains the sample time and the calculated specific activity as dpm/mg ('filtrate SA') and as fraction of dose/mg glucose ('SA/dose'). The final column of this table prints the ratio of ^3H

specific activity/ ^{14}C specific activity, as an indicator of the tracer ratio changes during the experiment.

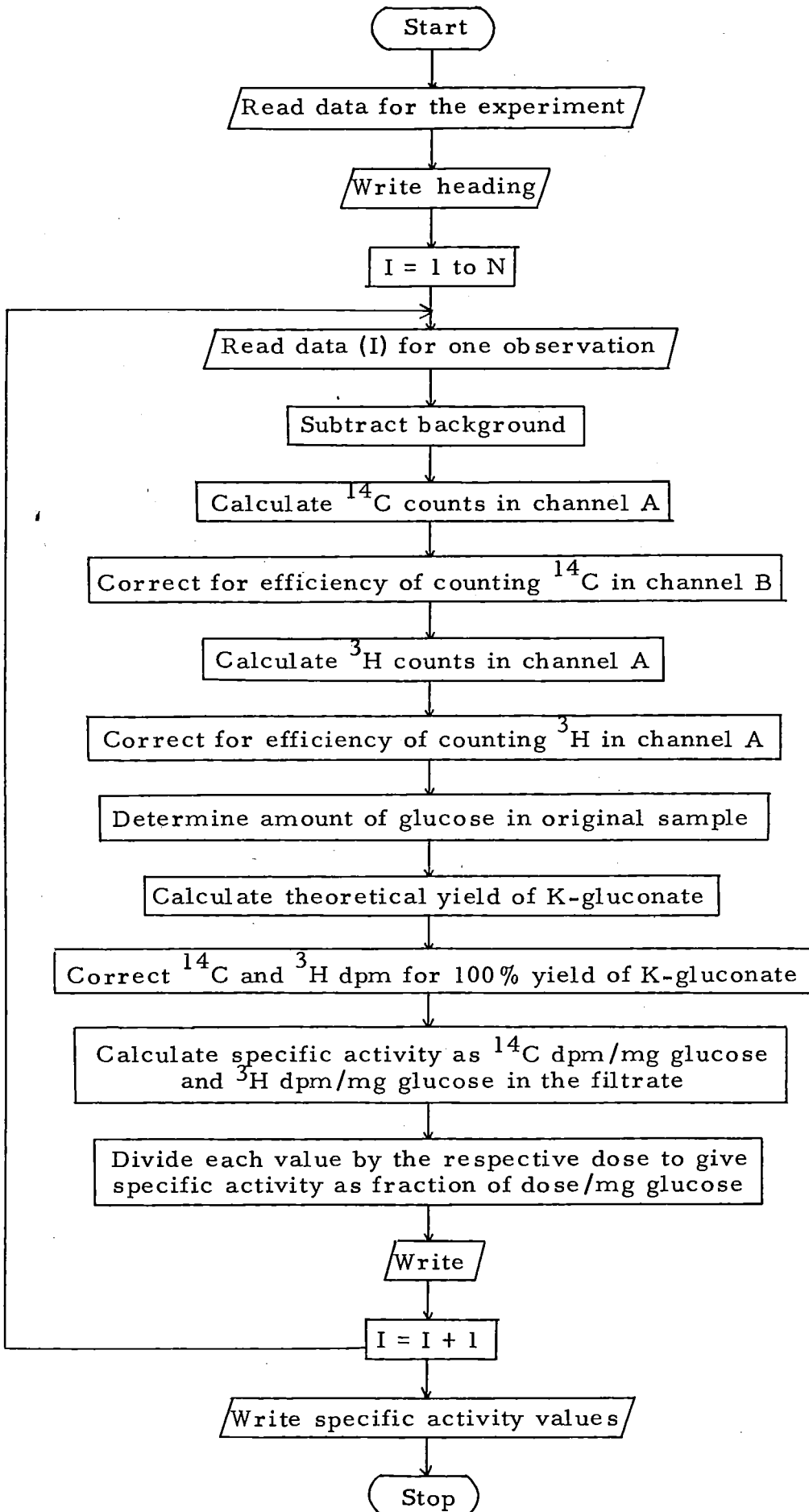


Fig 23: Flow chart for GLUCOSES78.

TABLE 33: GLUCOSESA78: Calculation of Glucose
Specific Activity.

a) Program Listing

```

MASTER GLUCOSESA78
DIMENSION F(50),DC(50),DT(50),SAC(50),SAT(50),RATIO(50),
*          PSAC(50),PSAT(50)
K=1
L=2
REAL M
INTEGER EXPT
100 READ (K,10) N,EXPT,BKA,BKB,CDOSE,TDOSE
10  FORMAT (2I5,2F5.1,2F12.0)
   IF (EXPT.LT.0) STOP
   WRITE (L,20) EXPT,BKA,BKB,CDOSE,TDOSE
20  FORMAT(12H1EXPT. NO.= ,I5/18H BACKGROUND CPM = ,F6.2,F8.2,
*18H CDOSE = ,F12.0,10X,7HTDOSE = ,F12.0//
*42H NO.  MG%G      MG%PG      MG K-G      VOL      ESR,6X,3HTCA,9X,2HCB,7X,
*2HCA,10X,2HTA,6X,4HEFCB,6X,4HEFTA//)
C
DO 50 I= 1,N
   READ(K,30) F(I), TCA, CB, G, M, V, S,PG
30  FORMAT (5F10.1,F5.1,F6.4,F6.2)
   IF (TCA.EQ.0) GO TO 50
C   B= BACKGROUND, K = COUNTS, S = ESR, E = EFFICIENCY,
C   C = 14C, T = 3H, F = TIME (MINS), D= DPM
C   * * * * *
C   SUBTRACT BACKGROUND COUNTS
C
   TCA = TCA - BKA
   CB = CB - BKB
C
   DETERMINE 14C COUNTS IN CHANNEL A AS X = CA/CB
C
   X = 3.47111906 - 4.498996108 * S + 1.692354905 * S ** 2
C
   CA = CB * X
C
   CONVERT COUNTS TO DPM USING EFFICIENCY CURVE OF 14C
   IN CHANNEL B
C
   EFCB = -0.05766976828 + 0.7415460384*S -0.1875925586*S**2
C
   DC(I) = CB/EFCB
C
C   3H COUNTS IN CHANNEL A = TOTAL COUNTS IN A - 14C COUNTS
C
   TA = TCA - CA
C
   CONVERT TO DPM
C
   EFTA = 0.013773569 + 0.1736364809 * S - 0.01146639054 * S**2
   DT(I) = TA / EFTA
C
C   * * * * *
C   CALCULATE SA AS DPM/MG GLUCOSE
C   1. DPM PRESENT:-
C       -DETERMINE THEORETICAL RECOVERY OF K-GLUCONATE FROM:
C           MW K-GLUCONATE / MW GLUCOSE : 1.30034
C       -CALCULATE QUANTITY GLUCOSE INITIALLY PRESENT FROM:
C           (100 MG CARRIER + (MG/ML IN FILTRATE * NO.ML TAKEN))

```

TABLE 33a (Continued)

```

C
C      -QUANTITY OF GLUCONATE FORMED = Q, WHERE
C
C       $Q = 1.30034 * (100 + \text{MG IN FILTRATE} * \text{ML TAKEN})$ 
C
C      -TOTAL DPM = (DPM / MASS GLUCONATE TAKEN) * Q
C
C      2.MG GLUCOSE PRESENT; GF
C       $GF = \text{MG\% FILTRATE} / 100 * \text{ML TAKEN}$ 
C       $SA = \text{TOTAL DPM} / \text{MG GLUCOSE}$ 
C      UNITS:  M = MASS GLUCOSE TAKEN (MG)
C              G = GLUCOSE IN FILTRATE (MG%)
C              V = VOLUME FILTRATE TAKEN (ML)
C
C       $GF = G / 100 * V$ 
C       $Q = 1.30034 * (100 + GF)$ 
C
C       $SAC(I) = DC(I) * Q / (M * GF)$ 
C       $SAT(I) = OT(I) * Q / (M * GF)$ 
C      SPECIFIC ACTIVITY DIVIDED BY DOSE TO GIVE SA/DOSE,
C       $PSAC(I) = SAC(I) / CDOSE$ 
C       $PSAT(I) = SAT(I) / TDOSE$ 
C       $RATIO(I) = SAT(I) / SAC(I)$ 
C
C
C      WRITE (L,40) I,G,PG,M,V,S,TCA,CB,CA,TA,EFCR,EFTA
40  FORMAT(1X,I2,3X,F5.2,3X,F5.2,6X,F4.1,3X,F4.1,2X,F7.4,2X,F9.1,
*2X,3(F9.1,2X),F4.2,5X,F4.2/)
50  CONTINUE
    WRITE (L,60)
60  FORMAT(1H1,51X,8HFILTRATE,26X,7HSA/DOSE,17X,2HSA/4H NO.,7X,4HTIME,
*9X,7H14C DPM,5X,6H3H DPM,6X,6H14C SA,7X,5H3H SA,14X,3H14C,10X,
*5H3H ,7X,12H3H/14C RATIO//)
    DO 70 J=1,N
    IF (F(J).EQ.0) GO TO 70
    WRITE (L,75) J,F(J),DC(J),OT(J),SAC(J),SAT(J),
*      PSAC(J),PSAT(J),RATIO(J)
75  FORMAT (1X,I3,4X,F8.2,5X,F9.1,3X,F9.1,3X,F9.1,4X,F10.1,6X, E11.5,
*      5X, E12.5,6X,F5.3/)
70  CONTINUE
    GO TO 100
  STOP
  END

END OF SEGMENT, LENGTH 396, NAME GLUCOSES78

```

TABLE 33 (Continued)

b) GLUCOSESA78 Output

EXPT..NO.= 12
 BACKGROUND CPM = 55.10 13.90
 DOSE = 545428669. TDOSE = 1432584528.

NO.	MGXG	MGXPG	MG K-G	VOL	ESR	TCA	CB	CA	TA	EFCB	EFTA
1	5.05	52.40	93.2	10.0	1.0840	65071.7	41398.1	24127.6	40944.1	0.53	0.19
2	5.12	53.10	86.5	15.0	1.0850	73880.7	47324.1	27542.2	46338.5	0.53	0.19
3	4.94	52.00	79.7	15.0	1.1040	63634.2	43152.1	24462.8	39171.4	0.53	0.19
4	5.01	52.70	98.3	15.0	1.0600	71675.7	44570.1	26907.6	44768.1	0.52	0.18
5	5.08	52.20	95.6	15.0	1.0530	66926.1	41687.1	25436.4	41489.7	0.52	0.18
6	4.89	51.10	78.9	10.0	1.0870	33917.7	21518.1	12487.9	21429.8	0.53	0.19
7	4.97	51.50	87.2	10.0	1.0880	34363.7	22010.9	12755.9	21607.8	0.53	0.19
8	5.13	52.40	86.2	15.0	1.0830	46087.1	29414.3	17167.7	28919.4	0.53	0.19

NO.	TIME	14C DPM	3H DPM	FILTRATE		SA/DOSE		SA 3H/14C RATIO
				14C SA	3H SA	14C	3H	
1	0.58	78743.4	217184.9	218650.9	403068.8	0.40013E-03	0.42102E-03	2.758
2	1.75	89958.0	245605.3	177436.0	484440.0	0.32472E-03	0.33821E-03	2.730
3	2.00	81058.7	204558.2	179798.6	453736.3	0.32904E-03	0.31677E-03	2.524
4	2.50	86110.8	242062.5	152715.7	429292.6	0.27948E-03	0.29970E-03	2.811
5	3.00	80918.5	225611.6	145542.1	405790.9	0.26639E-03	0.28330E-03	2.788
6	4.75	40851.7	113404.3	138356.5	384077.7	0.25320E-03	0.26814E-03	2.776
7	4.92	41760.8	114256.4	125923.3	344522.6	0.23049E-03	0.24052E-03	2.736
8	7.08	55984.7	153522.1	110595.9	303278.0	0.20240E-03	0.21173E-03	2.742

APPENDIX 3: Program WAVE

This program was written in Fortran by Dr C. F. Ramberg Jr, Ruakura ARC, Hamilton, to average the times of observation and specific activities of tracer experiments.

A listing of the program and a sample output are shown in Table 34. The program reads in the data from a disk file, WAVI, as $T(I, J)$ and $V(I, J)$ where T = time, V = specific activity, I = experiment number and J = the observation number within the experiment. The program will average up to 28 experiments with 24 observations in each.

The program calculates the mean time of each observation and the sum of squares about the mean. It takes the log of the specific activities and sums them, to determine the mean, and then determines the 95% confidence interval about this mean and the calculated values are transferred to a file, WAVO.

The output lists the mean times of observation $TM(I)$, the mean specific activities $VM(I)$, the sum of squares about the mean times $TSQ(I)$, and the 95% confidence interval about the specific activity, $VLOW$ and VUP .

TABLE 34: WAVE: Averaging Tracer Data.

a) Program Listing

```

00100      DIMENSION T(28,24),V(28,24),TSQ(24),VSQ(24),TM(24),VM(24),SD(24)
00200      OPEN(UNIT=20,FILE='WAVI')
00300      N=0
00400      DO20I=1,28
00500      DO10J=1,24
00600      READ(20,1000,END=99,ERR=999)T(I,J),V(I,J)
00630      10      CONTINUE
00700      N=N+1
00800      20      CONTINUE
00900      1000    FORMAT(6G)
01000      99      CONTINUE
01100      DO40J=1,24
01200      TM(J)=0.
01300      VM(J)=0.
01400      TSQ(J)=0.
01500      VSQ(J)=0.
01550      L=0
01600      DO28I=1,N
01650      IF (V(I,J).EQ.0) GO TO 28
01675      L=L+1
01700      TSQ(J)=TSQ(J)+T(I,J)*T(I,J)
01800      TM(J)=TM(J)+T(I,J)
01900      VSQ(J)=VSQ(J)+(ALOG(V(I,J)))*(ALOG(V(I,J)))
02000      VM(J)=VM(J)+ALOG(V(I,J))
02050      28      CONTINUE
02075      IF(L.LT.2) GO TO 30
02100      TSQ(J)=SQRT((TSQ(J)-TM(J)*TM(J)/L)/(L-1))
02200      VSQ(J)=SQRT((VSQ(J)-VM(J)*VM(J)/L)/(L-1))
02300      30      TM(J)=TM(J)/L
02400      40      VM(J)=VM(J)/L
02500      OPEN(UNIT=21,FILE='WAVO')
02600      DO50I=1,24
02700      VLOW=EXP(VM(I)-2*VSQ(I))
02800      VUP=EXP(VM(I)+2*VSQ(I))
02900      VM(I)=EXP(VM(I))
03000      50      WRITE(21,2000)TM(I),VM(I),TSQ(I),VLOW,VUP
03100      2000    FORMAT(5(2X,E20.5))
03200      999     CALL EXIT
03300      END

```

TABLE 34 (Continued)

b) WAVE Output.

TM(I)	VM(I)	TSQ(I)	VLOW	VUP
0.21500E+00	0.12634E-02	0.49497E-01	0.80484E-03	0.19831E-02
0.51000E+00	0.47145E-03	0.14142E-01	0.40142E-03	0.55370E-03
0.84000E+00	0.33404E-03	0.14142E-01	0.28771E-03	0.38784E-03
0.10500E+01	0.36305E-03	0.42427E-01	0.33054E-03	0.39875E-03
0.20000E+01	0.30780E-03	0.40000E+01	0.00000E+00	0.17014E+39
0.29650E+01	0.26183E-03	0.49497E-01	0.24046E-03	0.28509E-03
0.44250E+01	0.23327E-03	0.71418E+00	0.18458E-03	0.29480E-03
0.64650E+01	0.19778E-03	0.65761E+00	0.15437E-03	0.25340E-03
0.10000E+02	0.18093E-03	0.99004E-01	0.17716E-03	0.18478E-03
0.15040E+02	0.16173E-03	0.15556E+00	0.15771E-03	0.16585E-03
0.20210E+02	0.14454E-03	0.19797E+00	0.13361E-03	0.15636E-03
0.30545E+02	0.12912E-03	0.74245E+00	0.11725E-03	0.14219E-03
0.40130E+02	0.11740E-03	0.16104E+04	0.00000E+00	0.17014E+39
0.60085E+02	0.94127E-04	0.22097E-01	0.91333E-04	0.97007E-04
0.90085E+02	0.69911E-04	0.22097E-01	0.69577E-04	0.70246E-04
0.12006E+03	0.53961E-04	0.00000E+00	0.51244E-04	0.56823E-04
0.18017E+03	0.30173E-04	0.33146E+00	0.29988E-04	0.30360E-04
0.30035E+03	0.10367E-04	0.90210E+05	0.00000E+00	0.17014E+39
0.41550E+03	0.39210E-05	0.62650E+01	0.36640E-05	0.41959E-05
0.53800E+03	0.19314E-05	0.83862E+01	0.15198E-05	0.24545E-05
0.69600E+03	0.12197E-05	0.83867E+01	0.47203E-06	0.31515E-05
0.13945E+04	0.25939E-06	0.90933E+01	0.16382E-06	0.41072E-06
0.21035E+04	0.17378E-06	0.90967E+01	0.10463E-06	0.28863E-06
0.28110E+04	0.11159E-06	0.98043E+01	0.56815E-07	0.21917E-06

APPENDIX 4: Program MAPPER

This program maps from an exponential to a series model and was written by L. P. R. Danielson and Dr C. F. Ramberg Jr, Ruakura ARC, Hamilton, in Fortran to run interactively on a DEC-10 computer.

A copy of the program listing and output are shown in Table 35. For input, the program asks for the number of compartments (which equals the number of exponentials in the decay curve), the $L(0, J)$'s (slopes of the exponential curves) and the $S(0, J)$'s (the intercepts). The slopes and intercepts are entered as strings separated by commas.

The output consists of the calculated L 's of the series model.

TABLE 35: MAPPER: Mapping from Exponentials to Series Model

a) Program Listing

```

00100 C*****
00200 C
00300 C MAPPING FROM EXPONENTIALS TO SERIES MODELS
00400 C USED IN CONJUNCTION WITH SAAM
00500 C
00600 C PROGRAMMED BY: L.P.R. DANIELSON, RUAKURA A.R.C.
00700 C ALGORITHM SUPPLIED BY DR. C.F. HAMBERG, JR.
00800 C
00900 C*****
01000 C***** DEFINE ARRAYS
01100 C VECTOR OF LAMBDA'S (SUPPLIED BY USER)
01200 C VECTOR OF S(I,J)'S (SUPPLIED BY USER)
01300 C ARRAY OF A'S (CALCULATED)
01400 C ARRAY OF L'S
01500 COMMON D(20),S(20),A(20,20),L(20,20)
01600 REAL*8 D,S,A,L,F1,F2,F3,F4,V,X,S1,L01
01700 C***** READ IN LAMBDA'S AND S(I,J)'S
01800 10 TYPE 1000
01900 1000 FORMAT(/,1X,'MAPPING FROM EXPONENTIALS TO SERIES MODELS')
02000 TYPE 2000
02100 2000 FORMAT(/,1X,'NUMBER OF COMPARTMENTS IS ',S)
02200 ACCEPT 3000,N
02300 3000 FORMAT(G)
02400 IF(N.LT.0)GOTO999
02500 IF(N.LT.2)GOTO10
02600 IF(N.GT.20)GOTO10
02700 TYPE4000
02800 4000 FORMAT(1X,'ENTER L(0,J)'S SEPARATED BY COMMAS')
02900 ACCEPT5000,(D(I),I=1,N)
03000 5000 FORMAT(20G)
03100 TYPE6000
03200 6000 FORMAT(1X,'ENTER S(X,J)'S SEPARATED BY COMMAS')
03300 ACCEPT5000,(S(I),I=1,N)
03400 C
03500 C ***** INITIALIZE
03600 C
03700 S1=0.
03800 DO20J=1,N
03900 20 S1=S1+S(J)
04000 DO30J=1,N
04100 30 A(1,J)=S(J)/S1
04200 CALL F(F1,1,1,N)
04300 L(1,1)=-F1
04400 X=0.
04500 DO40J=1,N
04600 40 X=X+A(1,J)/D(J)
04700 L01=1./X
04800 L(2,1)=L(1,1)-L01
04900 C
05000 C***** MAIN LOOP
05100 C
05200 DO60I=2,N
05300 M=I
05400 M1=M-1
05500 M2=M-2
05600 M3=M+1
05700 C *3.1*
05800 CALL F(F1,M1,M,N)
05900 CALL F(F2,M2,M1,N)
06000 CALL F(F3,M1,M1,N)

```

TABLE 35a (Continued)

```

06100      CALL F(F4,M1,M2,N)
06200      L(M1,M)=(F1-L(N1,M2)*F2+L(M1,M1)*F3)/(L(M,M1)*F4)
06300  C      *3.2*
06400      DO50J=1,N
06500  50      A(M,J)=((L(M1,M1)-D(J))*A(M1,J)-L(M1,M2)*A(M2,J))/L(M1,M)
06600  C      *3.3*
06700      CALL F(F1,M,N,N)
06800      CALL F(F2,M1,M1,N)
06900      CALL F(F3,M,M1,N)
07000      L(M,M)=(F1-L(M,M1)*F2)/(-F3)
07100  C      *3.4*
07200  60      L(M3,M)=L(M,M)-L(M1,M)
07300  C
07400  C***** TYPE RESULTS
07500  C
07600      TYPE7000,L01
07700  7000      FORMAT(1X,'L( 0, 1)=' ,E13.6)
07800      DO70J=1,N-1
07900      1=J+1
08000      TYPE8000,J,I,L(J,I)
08100  8000      FORMAT(1X,'L( ',I2,', ',I2,', ' )=' ,E13.6)
08200  70      TYPE8000,I,J,L(I,J)
08300      GO1010
08400  999      STOP
08500      END
08600  C***** SUBROUTINES *****
08700      SUBROUTINE F(V,M,K,N)
08800      COMMON D(20),S(20),A(20,20),L(20,20)
08900      REAL*8 D,S,A,L,F1,F2,F3,F4,V,X,S1,L01
09000      X=0.
09100      DO10J=1,N
09200  10      X=X+(-D(J))*K*A(M,J)
09300      V=X
09400      RETURN
09500      END

```

TABLE 35 (Continued)

b) MAPPER Output.

```

--RUN SAAM-MAPPER
--
--* SAAM-MAPPER 06 SEP 78 15:48:13
--
--MAPPING FROM EXPONENTIAL TO SERIES MODELS
--
--NUMBER OF COMPARTMENTS IS ? 5
--ENTER L(C,J)'S SEPARATED BY COMMAS
--? 0.8468559,0.1003898,0.1114290E-1,0.3825455E-2,0.1245544E-3
--ENTER S(X,J)'S SEPARATED BY COMMAS
--? 0.1136395E-3,0.9358850E-4,0.9878646E-4,0.4032066E-5,0.3651224E-6
--
--L(-C,1) = 2.39436 E-2
--L(-1,2) = 0.460594
--L(-2,1) = 0.319948
--L(-2,3) = 6.10893 E-2
--L(-3,2) = 0.086492
--L(-3,4) = 1.16446 E-3
--L(-4,3) = 5.29354 E-3
--L(-4,5) = 8.52527 E-4
--L(-5,4) = 2.4215 E-3
--
--NUMBER OF COMPARTMENTS IS ? 5
--ENTER L(C,J)'S SEPARATED BY COMMAS
--? 0.2319529,0.463608E-1,0.2316844E-1,0.1213068E-1,0.1033931E-2
--ENTER S(X,J)'S SEPARATED BY COMMAS
--? 0.1161400E-3,0.4117740E-4,0.6559601E-4,0.7701795E-4,0.3376271E-6
--
--L(-C,1) = 2.75584 E-2
--L(-1,2) = 0.135508
--L(-2,1) = 7.66895 E-2
--L(-2,3) = 2.96084 E-2
--L(-3,2) = 1.30793 E-2
--L(-3,4) = 1.44864 E-2
--L(-4,3) = 7.1959 E-3
--L(-4,5) = 1.95087 E-3
--L(-5,4) = 8.56954 E-3
--
--NUMBER OF COMPARTMENTS IS ? 5
--ENTER L(C,J)'S SEPARATED BY COMMAS
--? 0.2906087E+1,0.2149166,0.2257550E-1,0.7858816E-3,0.1144551E-1
--ENTER S(X,J)'S SEPARATED BY COMMAS
--? 0.4458098E-3,0.1241864E-3,0.1018157E-3,0.2884825E-6,0.5624625E-4
--
--L(-C,1) = 6.92174 E-2
--L(-1,2) = 1.0666
--L(-2,1) = 1.75024
--L(-2,3) = 0.130879
--L(-3,2) = 0.116032
--L(-3,4) = 1.21148 E-2
--L(-4,3) = 6.0833 E-3

```

APPENDIX 5: Animal Data

Individual sheep data for glucose tolerance are listed in Table 36, and hepatocyte glucose production rates in Table 37.

TABLE 36: Glucose Tolerance of Ewes.

Group	Expt.	$T_{\frac{1}{2}}$ (min)	I' (μ U/ml)	R (μ U/ml.min)
a) Twin Pregnant				
Susceptible	12	44.9	27.3	1226
Live	13	42.4	48.0	2035
	27	49.3	34.5	1701
	32	59.0	32.5	1918
	34	36.3	44.0	1597
	17	59.0	54.5	3216
	18	60.2	43.3	2607
Non-susceptible				
Live	5	41.8	30.5	1275
	24	32.7	55.5	1814
	28	43.6	37.0	1613
	33	51.0	26.3	1341
	38	21.5	37.0	796
	40	34.6	21.0	727
Dead	9	77.2	6.3	486
	14	41.8	20.5	857
	39	35.4	51.5	1823
b) Non-Pregnant				
Fed Daily	2	24.4	88.3	2141
	4	54.9	83.3	4231
Fed Hourly	1	32.4	51.8	1682
	3	38.6	30.2	1106

Plasma glucose and insulin concentrations were measured for two hours after an intravenous injection of glucose (0.4 g/kg) and glucose tolerance ($T_{\frac{1}{2}}$), extravascular insulin concentration, when plasma glucose was 100 mg/dl above basal (I') and insulin resistance (R) were determined. The pregnant ewes were classed, after a period of starvation, as susceptible or non-susceptible to pregnancy toxæmia, with live or dead lambs in utero at slaughter.

TABLE 37: Hepatocyte Glucose Production of Pregnant Ewes.

Ewes were susceptible (S) or non-susceptible (NS) to pregnancy toxaemia, with live (L) or dead (D) lambs in utero at slaughter.

Hepatocytes were isolated from the caudate lobe by perfusion with collagenase, and incubated for 30 min with substrate (10 mM) and glucagon (10^{-7} or 10^{-8} M).

B - Basal, P - Propionate, L - Lactate
G - Glutamine, A - Alanine, Gc - Glycerol
gn - glucagon.

a) 1977

Group	Ewe	Glucose Production ($\mu\text{mol/g/min}$)											
		B	10^{-7}gn + B	10^{-8}gn + B	P	10^{-7}gn + P	10^{-8}gn + P	L	10^{-8}gn + L	G	10^{-8}gn + G	+ A	10^{-8}gn + A
S-L	294	-0.05	-0.03	-0.05	-0.04	-0.08	-0.08	-0.09	-0.04	-0.08	-0.13	-0.03	-0.07
	349	0.45	0.71	0.60	1.07	0.78	0.96	1.05	0.98	0.69	0.68	0.56	0.65
	288	0.02	-0.12	0.09	-0.01	0.34	0.09	0.01	0.03			0.16	0.08
	356	0.12	0.03	0.07	0.05	0.05	0.00	0.03	0.03			-0.02	-0.03
	371	0.02	-0.15	0.04	0.00	-0.13	-0.04	-0.06	-0.15	-0.06	-0.01	-0.10	-0.13
	322	0.07	-0.22	0.09	0.16	-0.22	0.02	-0.04	-0.13	-0.60	-0.71	-0.24	-0.53
	363		0.03	0.10	-0.18	0.34	0.42	0.00	0.03	0.24	0.39	-0.15	0.31
NS-L	114	0.86	1.52	1.62	1.80	2.14	2.30	1.73	2.14				
	357	0.90	2.17	1.88	1.64	2.15	1.70	2.06	2.09			1.80	2.00
	313	0.25	0.88	0.56	0.38	0.32	0.29	0.52	0.42			0.29	0.48
	390	0.43	0.83	0.79	0.51	1.04	1.14	0.91	1.09	0.43	0.77	0.33	0.61
	330	0.39	0.44	0.52	0.82	0.63	0.80	0.93	0.90	0.46	0.49	0.44	0.47
	335	1.07	2.48	2.97	2.17	2.67	3.13	2.12	2.63	1.42	2.48	1.38	2.27
NS-D	299	1.32	4.81	4.38	8.58	9.23	7.04	6.11	3.02	8.51	9.27	2.31	7.56
	368	0.99	5.24	6.67	2.27	4.14	7.63	2.05	5.67	2.28	5.69	2.09	4.94
	4	0.77	5.06	3.80	3.67	5.53	5.49	1.22	5.18	5.12	4.63	3.72	3.13

TABLE 37 (Continued)

b) 1978

Group	Ewe	Glucose Production ($\mu\text{mol/g/min}$)									
		B	$10^{-8}\text{gn} + \text{B}$	P	$10^{-8}\text{gn} + \text{P}$	L	$10^{-8}\text{gn} + \text{L}$	A	$10^{-8}\text{gn} + \text{A}$	Gc	$10^{-8}\text{gn} + \text{Gc}$
S-L	38	0.40	0.32	1.52	1.19	1.40	1.13	0.75	0.75	0.44	0.23
	26	0.13	0.08	0.41	0.16	0.43	0.16	0.15	0.07	0.19	0.05
	178	0.08	0.13	0.12	0.12	0.18	0.11	0.13	-0.19	0.13	0.20
S-D	155	0.00	-0.18	-0.12	-0.19	-0.02	-0.18	-0.03	-0.18	-0.02	-0.16
	6	0.69	0.73	1.06	1.17	0.85	0.96	0.82	0.81	0.41	0.20
	84	-0.09	-0.05	-0.01	-0.01	-0.08	0.01	-0.02	0.02	-0.06	0.01
	172	1.00	1.14	1.49	1.64	1.22	0.98	0.87	1.27	0.94	0.66
NS-L	193	0.02	0.12	0.08	0.16	0.07	0.11	0.12	0.07	0.04	-0.02
NS-D	107	6.61	8.18	7.97	9.42	6.28	8.06	6.89	7.98	6.08	7.86
	132	-0.05	0.04	0.08	0.04	0.07	0.08	0.11	0.12	0.07	0.11
	57	1.96	2.01	2.26	2.81	2.29	2.31	0.81	2.27	1.05	0.53